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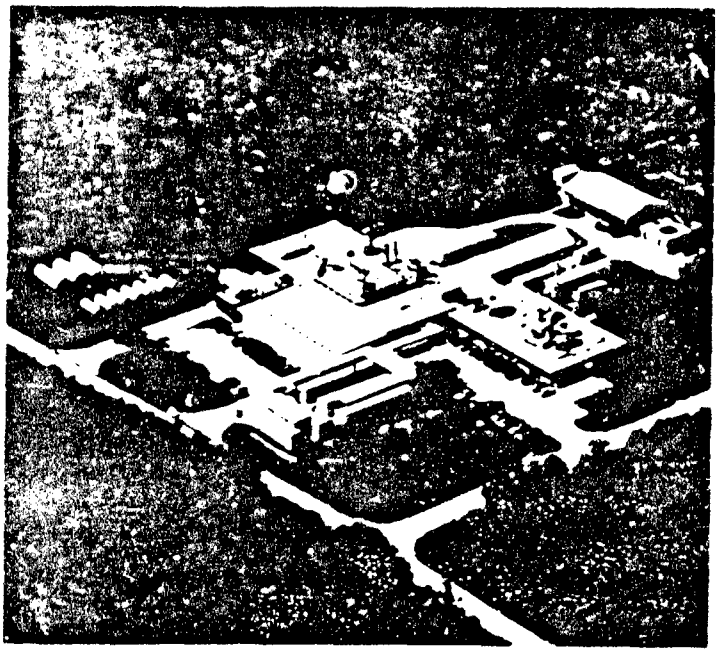
FINAL REPORT



Task 89-03: Test Up To 20 Candidate Topical Protectants

To

U.S. Army Medical Research and Development Command

Contract No. DAMD17-89-C-9050

February 1, 1992

Battelle

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Fourteen candidate topical skin protectants (TSPs) were examined in a multiple-phase testing regimen that included a variety of testing models designed to assess different aspects of TSP efficacy. Phase 1 initial efficacy tests were performed using both in vitro and in vivo models. The Phase 1 in vitro model involved determining the penetration profile of a challenge dose of chemical surety materiel (CSM), either soman (GD), VX, or thickened soman (TGD), through a 0.1 mm thick film of TSP layered between two disks of synthetic membrane. The in vivo models were performed in New Zealand white rabbits pretreated with 0.1 mm thick layers of TSP. Following topical application of an organophosphonate challenge (TGD) on rabbits, the end point for determining TSP efficacy was erythrocyte acetylcholinesterase inhibition profiles. Following topical application of a vesicant challenge (HD), the end point for TSP efficacy was the size of the lesion resulting after prescribed exposure periods.

Phase 2 consisted of in vivo testing only and differed from Phase 1 in that the candidate TSPs were subjected to water and time stress before challenging with HD

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or TCD. Phase 3 further expanded Phase 1 testing by challenging the TSP with GD, VX, or combined HD/Levisite (L). Polyethylene glycol (MW = 540 daltons, PEG 540) was used as a quality control TSP and a reference standard throughout the task.

TSPs ranged from completely ineffective to hightly effective against specific CSM challenges. Three TSPs, ICD Nos. 1465, 1511, and 1536, were selected for testing in Phases 2 and 3.

FINAL REPORT

A Medical Research and Evaluation Facility (MREF) and Studies Supporting the Medical Chemical Defense Program

on

TASK 89-03: TEST UP TO 20 CANDIDATE TOPICAL PROTECTANTS

to

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21702-5012

February 1, 1992

Contract No. DAMD17-89-C-9050

Dr. David W. Hobson
Mr. Thomas H. Snider

Battelle Columbus Operations
505 King Avenue
Columbus, Ohio 43201-2693

FINAL REPORT

on

TASK 89-03
TEST UP TO 20 CANDIDATE TOPICAL PROTECTANTS

to

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND

February 1, 1992

David W. Hobson, Ph.D., D.A.B.T. Date
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EXECUTIVE SUMMARY

Fourteen candidate topical skin protectants (TSPs) were examined in a multiple-phase testing regimen that included a variety of testing models designed to assess different aspects of TSP efficacy. Phase 1 initial efficacy tests were performed using both *in vitro* and *in vivo* models. The Phase 1 *in vitro* model involved determining the penetration profile of a challenge dose of chemical surety materiel (CSM), either soman (GD), VX, or thickened soman (TGD), through a 0.1 mm thick film of TSP layered between two disks of synthetic membrane. The *in vivo* models were performed in New Zealand White rabbits pretreated with 0.1 mm thick layers of TSP. Following topical application of an organophosphonate challenge (TGD) on rabbits, the end point for determining TSP efficacy was erythrocyte acetylcholinesterase inhibition profiles. Following topical application of a vesicant challenge (HD), the end point for TSP efficacy was the size of the lesion resulting after prescribed exposure periods.

Phase 2 consisted of *in vivo* testing only and differed from Phase 1 in that the candidate TSPs were subjected to water and time stress before challenging with HD or TGD. Phase 3 further expanded Phase 1 testing by challenging the TSP with GD, VX, or combined HD/Lewisite (L). Polyethylene glycol (MW = 540 daltons, PEG 540) was used as a quality control TSP and a reference standard throughout the task.

TSPs ranged from completely ineffective to highly effective against specific CSM challenges. Three TSPs, ICD Nos. 1465, 1511, and 1536, were selected for testing in Phases 2 and 3.

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TEST UP TO 20 CANDIDATE TOPICAL PROTECTANTS

1.0 INTRODUCTION

The U.S. Army Medical Research and Development Command (USAMRDC) via the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD) is currently evaluating candidate topical skin protectants (TSPs) for their efficacy against various chemical surety materiel (CSM) challenges. Task 89-03 was conducted at Battelle's Medical Research and Evaluation Facility (MREF) to examine the protective effectiveness of TSPs submitted by USAMRICD for testing. Materials and methods employed in this study are detailed in MREF Protocol 58, supplied as Appendix A to this report and entitled, "Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HL." The task consisted of three test phases (see diagram in Appendix A), which included both in vitro and in vivo tests conducted under a variety of test conditions and CSM challenges. Upon completion of each phase of testing, candidate TSPs were statistically ranked from most to least effective relative to each CSM challenge. The objective of this task was to provide information to USAMRICD scientists to be used for identification of efficacious candidate TSPs for transition to more advanced testing and field evaluations.

Letter reports of the results were presented to USAMRDC as each phase of screening was completed. Based on the letter reports (included as appendices B, C, and D of this report), decisions to transition TSPs from one phase to the next for further testing was made by USAMRICD investigators with consultation from MREF personnel. Thus, of the 14 TSPs tested in Phase 1, five TSPs were initially transitioned for testing in Phase 2. However, at the further direction of USAMRICD, only three of these TSPs were fully tested in Phase 2 and were then transitioned to Phase 3. The other two TSPs transitioned to Phase 2 were, therefore, only partially tested.

In addition to the above work, five special studies associated with assessing the in vivo efficacy of various lots of ICD No. 1536 were performed under Phase 1 of MREF Task 89-03 to meet additional test requirements needed to answer questions raised during Operations Desert Shield and Desert Storm.

A summary of findings from each special study is presented in Section 3.6 of this report.

2.0 MATERIALS AND METHODS

Polyethylene glycol with a mean molecular weight of 540 daltons (PEG 540, or Carbowax, from Union Carbide) was included in all phases as a TSP control. Stringent quality control processes based on the day-to-day efficacy of PEG 540 were maintained throughout each phase of study to control for variable experimental conditions.

All CSM was supplied by USAMRICD. HL was formulated at the MREF as 75 percent HD and 25 percent L by volume. Purities of GD, GD in TGD, VX, and HD were assessed by Battelle chemists prior to use on study. The mean purity and range of acceptable purities (95 percent confidence limits) used in Task 89-03 are listed in Table 1. Volumes of GD, TGD, and VX for application on rabbit backs were adjusted for purity.

TABLE 1. RANGE OF ACCEPTABLE CSM PURITIES (percent) USED IN TASK 89-03

CSM	Mean	Standard Deviation	Lower Limit	Upper Limit
GD	88.2	2.2	83.8	96.2
TGD	82.6	5.4	71.8	93.4
VX	76.8	3.8	69.2	84.4
HD	87.7	2.5	82.7	92.7

A list of the 14 TSPs tested in Task 89-03, identified by ICD No., MREF No., product name, and manufacturer is presented in Table 2.

TABLE 2. TOPICAL SKIN PROTECTANT (TSP) IDENTIFICATION CROSS-REFERENCE

ICD No.	MREF No.	Product Name	Manufacturer
-	PEG 540	Polyethylene glycol, 540 daltons	Union Carbide, Danbury, CT 06817
1463	DP56-89	Krytox® Fluorinated Greasc	DuPont, Wilmington, DE 19099
1465	DP41-89	Krytox® Fluorinated Grease TLF-7768A	DuPont, Wilmington, DE 19899
1466	DP42-89	Krytox® Fluorinated Grease TLF-7768B	DuPont, Wilmington, DE 19899
1467	DP43-89	Krytox® Fluorinated Grease TLF-7768C	DuPont, Wilmington, DE 19899
1469	DP45-89	Krytox® Fluorinated Grease TLF-7770	DuPont, Wilmington, DE 19899
1509	BM53-89	Barrier Biocream®	Biomedic, Busnago, Italy
1511	MA54-89	Fomblin® Perfluorinated Grease	Montefluos Grappc Ausimont, Milano Italy
1536	MS55-89	Multishield® Skin Cream	Interpro, Inc., Haverhill, MA 01831
1621	BC61-89	unknown[1]	unknown
1623	MS163-89	MSI	unknown
1689	3M66-90	Fluorochemical L-12079	3M Corp., St. Paul, MN 55144
1690	3M67-90	Fluorochemical L-12078	3M Corp., St. Paul, MN 55144
1691	3M65-90	Fluorochemical L-12080	3M Corp., St. Paul, MN 55144
1692	3M64-90	Fluorochemical L-12081	3M Corp., St. Paul, MN 55144

[1]Information about the product and manufacturer for some materials was not submitted to Battelle.

2.1 Test Animals

New Zealand White rabbits were chosen as the in vivo test model for this study on the basis of the extensive MREF data base and experience for percutaneous applications of CSM and TSPs to the species. Male, specific pathogen-free (SPF) rabbits were homogeneously assigned to treatment groups based on body weights (2.0 to 4.0 kg). Rabbits were purchased from Hazleton Laboratories, Kalamazoo, MI. Rabbits were quarantined at either the MREF or at the Battelle Animal Resources Facility, 505 King Avenue, Columbus, OH, before being transported to the MREF. Upon receipt at the MREF, the animals were weighed, sexed, and observed for signs or symptoms of disease during a quarantine period of at least 7 days. Positive identification of animals throughout each study was maintained by ear tattoo.

Animals transported to the MREF from the King Avenue facility were acclimated for at least 24 hr prior to being placed on study. At both facilities, housing was individual in stainless-steel, slotted cages equipped with automatic watering systems. Humidity was programmed and maintained at 50 percent (± 10 percent) and temperature at 21 C (± 3 C). Fluorescent lighting was maintained at a light/dark cycle of 12 hr each per day. Purina Certified Rabbit Chow and water were available at all times during quarantine and holding. During the 24-hr test period, animals were given free access to water, but were not given rabbit chow while in the treatment stanchions. No contaminants which would interfere with the results of the study are known to be present in the water or feed.

Rabbits were given 5.0 mg/kg (20 mg/mL) xylazine and 35.0 mg/kg (100 mg/mL) ketamine by intramuscular injection prior to the marking of test sites and TSP application.

2.2 Facilities

Battelle's Animal Resources Facilities have been registered with the U.S. Department of Agriculture (USDA) as a research facility (Number 31-21) since August 14, 1967, and are periodically inspected in accordance with the provisions of the Federal Animal Welfare Act. Battelle's statement of assurance regarding the Department of Health and Human Services policy on

humane care of laboratory animals was accepted by the Office of Protection from Research Risks, National Institutes of Health, on August 27, 1973. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 86-23) and/or in the regulations and standards that are promulgated by the Agricultural Research Service, USDA, pursuant to the Laboratory Animals Welfare Act of August 24, 1966 as amended.

On January 31, 1978, Battelle Memorial Institute received full accreditation of its animal care program and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. The MREF is a part of the facilities granted full accreditation.

2.3 Experimental Design

Phase 1, designated the initial efficacy phase, was conducted in two parts, one *in vitro*, and one *in vivo* involving male New Zealand White rabbits. The purpose of Phase 1 was to determine where a given TSP would rank for initial efficacy relative to other TSPs using GD, TGD, or VX (*in vitro*) and HD or TGD (*in vivo*) as challenge agents. HD and TGD were selected for use in Phases 1 and 2 *in vivo* tests because both present substantial cutaneous hazards, and represent the two broad classes of chemical agents (i.e., vesicants and nerve agents) for which TSPs should be efficacious.

Phase 2, designated the functional testing phase, was conducted to determine how TSPs transitioned from Phase 1 would be affected by factors encountered in field use, i.e., time of wear and environmental moisture.

Phase 3, designated the advanced efficacy phase, was conducted to determine how successful candidate TSPs from the previous phases protect against a wider range of challenge agents, namely GD, VX, and HL. HL was defined for this study as a mixture of 75 percent HD and 25 percent L by volume.

2.3.1 Phase 1, Initial Efficacy, In Vitro

During the in vitro part of Phase 1, a 0.5 µL volume of nerve agent (GD, TGD, or VX) was applied on a triple-layer test assembly that was positioned in a Teflon® Flow-Thru Diffusion Cell (Crown Glass, Somerville, NJ) maintained at 37 C in a water-heated receptacle. The test assembly was made of a disk of candidate TSP sandwiched between either two, 0.25 mm thick disks of dimethylpolysiloxane (Silastic®, Dow Corning Corp., Midland, MI) for tests involving GD and TGD, or two, 0.13 mm thick layers of Durapore® (Millipore Corp, Bedford, MA) for tests involving VX. The inner disk of TSP was 0.1 mm thick and 9 mm in diameter, and was held in place by a 0.1 mm thick, hardened steel shim. Receptor fluid flowing through the cell at 0.15 mL/min was collected in 5 min fractions. The penetration of nerve agent through the TSP was detected by inhibition of eel acetylcholinesterase (AChE, 20 U/mL, Sigma No. C-2629) in the receptor fluid fractions. The end point in these studies was the time (up to 2 hr) required after dosing to reach 25, 50, and 75 percent inhibition of the AChE relative to a parallel control penetration cell in which nerve agent was not dosed. The nominal sample size for statistical contrasts among TSPs was 12 cells per TSP.

2.3.2 Phase 1, Initial Efficacy, In Vivo

The in vivo part of Phase 1 was performed using New Zealand White rabbits in a percutaneous exposure model with TGD and HD as challenge agents. The rabbits were anesthetized (5 mg/kg xylazine and 35 mg/kg ketamine), and their dorsa from withers to rump were shaved with electric clippers.

In the Phase 1 TGD test, a 24-gauge, 1 mL catheter was inserted into the central artery of each rabbit ear for serial blood collection. A 3.81-cm diameter pretreatment area was marked on the shaved dorsum with a felt-tip pen. TSP was evenly applied at an average rate of 0.01 mL/cm^2 (equivalent to 0.1 mm thickness) within the circle. A rubber "O" ring with a 3.81-cm inside diameter was affixed with cyanoacrylate glue around the pretreatment area. The rabbits remained undisturbed for 1 hr before receiving the TGD challenge. Blood samples were collected from each rabbit before and after the TSP application. At the end of the 1-hr waiting period, TGD was applied at the

predetermined LD_{50} (3.35 mg/kg) to the TSP application area, and blood samples were collected at 30, 60, and 120 min after dosing. In one of the three replicate tests, the rabbits were removed from their tie-down boards, transferred to stainless steel stanchions, and remained in the dosing hood overnight. A 24-hr post-CSM exposure blood sample was collected from this replicate group of rabbits. The end point in these studies was erythrocyte AChE activity expressed relative to the pre-TSP application baseline level in each rabbit (RA values). The nominal sample size, typically reached after three days of testing, was 24 rabbits per TSP.

On each day of testing, a process control group of eight rabbits was pretreated with PEG 540 and dosed with 3.35 mg/kg of TGD. If any of the RA means at 30, 60, or 120 min in these rabbits was outside a critical range, which was specified as the historical mean plus or minus three standard deviations, then all data from that test day were excluded from the study. The baseline absolute AChE activity levels were also subjected to process quality control, but on an individual animal basis. That is, any rabbit with a baseline level outside a critical range (the historical mean plus or minus three standard deviations) was excluded from the study.

In the Phase I HD test, TSP was evenly applied at an average rate of 0.01 mL/cm^2 to three, 2.5-by-5-cm rectangular areas on one side of the shaved rabbit dorsum. Either a second TSP, or PEG 540 was similarly applied at three sites on the other side. A 1 µL volume of HD was applied to the center of each of the six sites and at an untreated control site per rabbit. CSM applications were made carefully so as to not alter the even spreading of TSP at the dose site with the dosing apparatus.

The HD test sites were decontaminated in pairs, at 1, 2, and 4 hr after HD dosing. The objective of sequential decontaminations was to halt the penetration of HD over a range of exposure periods, thus obtaining a graded response. Decontamination consisted of wetting an applicator (a plastic-backed paper pad wrapped around a tongue depressor) with 3 mL of a 5 percent sodium hypochlorite (NaOCl) solution, holding it on the dose site for 10 sec, and turning over the applicator and holding it to the dose site for another 10 sec. Water rinses (3 mL of distilled water placed on an applicator and held on the dose site for 10 sec per side) were performed twice at each site after decontamination. All traces of NaOCl were adequately removed by this method,

since there was no evidence of dermal irritation at portions of the dose site peripheral to the area resulting from HD exposure. The untreated control site was decontaminated at 24 hr after dosing. MREF Protocol 58 specified a repeat decontaminatior at each site, but this was not performed throughout the study. However, this protocol deviation had no apparent effect on the lesion area assessments.

At 20-24 hr after dosing, each animal was given a 2.0-mL intramuscular injection, in each thigh, of a 3 percent suspension of Trypan blue dye in saline. At approximately 24 hr after dosing, the resulting lesion area was estimated, and a lesion area ratio (LAR) relative to the untreated control site lesion area for that rabbit was calculated for each pretreated dose site. The nominal sample size, typically reached over three days of testing, was 24 rabbits per TSP.

On each day of testing, the right side of one group of eight rabbits was pretreated with PEG 540 and dosed with HD. If the LAR means for all three PEG 540-pretreated sites were outside a critical range, i.e., the historical mean plus or minus three standard deviations, then all data from that test day were excluded from the study. The untreated control site lesion areas were also subjected to process quality control, but on an individual animal basis. That is, any animal with an untreated control site lesion area outside a critical range (the historical mean plus or minus three standard deviations) was excluded from the study.

2.3.3 Phase 2, Functional Testing

The purpose of Phase 2, designated the functional testing phase, was to determine the relative performance of TSPs against TGD or HD following TSP stressing with either water or time of wear. In both the water and time stress tests, rabbits were prepared as in the Phase 1 *in vivo* studies, including clipping, anesthesia, markings, collection of baseline blood samples (for TGD challenges) and application of TSPs.

In water stress tests, TSP was applied within each pretreatment area, and either a 3.81-cm diameter, 5-cm tall plastic cylinder (for TGD challenges) or a four-sided, 2.5-by-5-cm box (for HD challenges) was placed around it. Five sequential aliquots of distilled water were dispensed into

the container (cylinder or box), which was held firmly in place for 10 sec. At the end of each 10-sec period, the container was lifted up, and the water was allowed to flow off the rabbit's back. The total volume of water used at each pretreatment site was 500 times the volume of TSP applied. For TGD challenges, the test consisted of five, 11-mL aliquots. For HD challenges, the test consisted of five, 13-mL aliquots. After the final, fifth water stressing, the pretreatment site remained undisturbed for 1 hr. PEG 540 control sites and untreated control sites were not water-stressed. All other aspects of the water stress tests were identical to the Phase 1 *in vivo* tests.

In time stress tests, TSP applications were made on pretreatment areas and remained undisturbed for 4 hr between the last application on a rabbit and the beginning of CSM dosing for that rabbit. All other aspects of the time stress tests were identical to the Phase 1 *in vivo* tests, including end point measurements and quality control procedures; PEG 540 was not stressed in any tests of Task 89-03.

2.3.4 Phase 3, Advanced Efficacy

The objective of Phase 3, designated the advanced efficacy phase, was to determine the relative efficacy of each TSP against a broader spectrum of possible challenge agents. Phase 3 was conducted identically to Phase 1 *in vivo* tests except that either GD or VX was substituted for TGD and HL was used in place of HD. The challenge doses of GD and VX administered were 1.35 mg/kg and 0.3 mg/kg, respectively, equivalent to one and 10 LD_{50}s, respectively, on unprotected rabbit backs. The dose of HL used was 1.0 µL per site. Separate historical data bases were established and maintained for both PEG 540-pretreated sites and untreated control sites dosed with either GD, VX, or HL.

2.4 Statistical Analyses

All calculations were performed on a DEC VAX using the Statistical Analysis System (SAS, Cary, NC).

2.4.1 Phase 1 In Vitro and In Vivo Statistical Procedures

Univariate statistics were tabulated for end point parameters (RAs for organophosphonate challenge studies, and LARs for HD challenge studies) by TSP and exposure period. Univariate statistics were tabulated for Scores by TSP.

For each CSM, TSPs were ranked and compared among all possible combinations of TSP pairs. Contrasts were made with the SAS general linear models procedure (PROC GLM) with Tukey's multiple comparisons option. The experiment-wise error rate was controlled at 0.05 with a Bonferroni adjustment. That is, a pair of TSPs was considered statistically different if the p value was less than 0.05 divided by the number of paired comparisons.

For in vitro tests, times to 25, 50, and 75 percent relative AChE inhibition in receptor fluid were submitted for statistical analysis. In addition, the means or "Scores" for times across those three inhibition levels for a given test cell were also analyzed.

For tests involving an organophosphonate challenge, RAs at 30, 60, and 120 min after dosing were submitted for statistical analysis. In addition, the means or "Scores" for RAs averaged across those three blood sample times for a given animal were also analyzed.

For tests involving HD challenges, lesion area ratios (LARs) obtained at 1, 2, and 4 hr after dosing were submitted for statistical analysis. The means or "Scores" for LARs averaged across those three exposure times for a given animal were also statistically analyzed.

For tests involving an organophosphonate challenge, paired t tests between baseline (65 min before dosing) and pre-dose (5 min before dosing) RAs were calculated to detect any significant ($p < 0.05$, two-sided) effect of wearing each TSP for 1 hr. Also, paired t tests between 120-min and 24-hr RAs were calculated to detect any significant ($p < 0.05$, two-sided) recovery in AChE activity or delayed penetration of CSM. The latter paired t test was limited to eight-rabbit replicates held overnight from each of the three replicates conducted for each TSP test.

2.4.2 Phase 2 Statistical Procedures

Univariate statistics were tabulated for end point parameters (RAs for TGD challenge studies and LARs for HD challenge studies) by phase, TSP, and exposure period.

For both TGD and HD challenge studies, the effect of either water or time stressing of TSPs was assessed by contrasting end point results (RAs or LARs) from Phase 2 with those from Phase 1. Student's two-sided t test was used at the 5 percent level. For HD studies, LARs determined after 1- and 2-hr exposures were contrasted (the Phase 1, 4-hr LAR data were not used). For TGD studies, RAs determined at all blood samples (-65, -5, 30, 60, and 120 min, and 24 hr) were contrasted. Contrasts were not made between water-stressed results and time-stressed results.

2.4.3 Phase 3 Statistical Procedures

The same analytical procedures detailed for Phase 1 *in vivo* data from studies using TGD and HD challenges were employed for Phase 3 studies using GD, VX, and HL challenges.

3.0 RESULTS

3.1 Phase 1, Initial Efficacy In Vitro

All work accomplished under Phase 1 is presented in a letter report dated 17 August 1990 and attached as Appendix B, "Letter Report on Phase 1, Initial Efficacy Testing." At the time of the report, TSPs were referred to as candidate topical protectants (CTPs). Both acronyms refer to candidate materiel that may be applied to skin before topical exposure to CSM, and either retard or prevent CSM penetration into skin. In later studies, however, TSP became the preferred acronym for these materiel. Page number references to data in appended reports appear on the bottom center of the referenced pages.

Of the 14 TSPs tested against GD penetration in the *in vitro* flow cell portion of Phase 1, four TSPs (ICD Nos. 1689, 1463, 1511, and 1691) were

statistically ($p < 0.05$, Tukey's multiple comparisons test) better than PEG 540, nine TSPs (ICD Nos. 1465, 1469, 1467, 1692, 1466, 1690, 1621, 1536, and 1509) were statistically indistinguishable from PEG 540, and one TSP (ICD No. 1623) was worse than PEG 540 (see page B-5).

Against a TGD challenge, four TSPs (ICD Nos. 1465, 1467, 1469, and 1463) were statistically better than PEG 540, eight TSPs (ICD Nos. 1511, 1466, 1691, 1623, 1621, 1536, 1509, and 1692) were statistically indistinguishable from PEG 540, and two TSPs (ICD Nos. 1690 and 1689) were worse than PEG 540 (see page B-7). Notably, the best TSP against GD, ICD No. 1690, was also the worst TSP against TGD.

Against a VX challenge, seven TSPs (ICD Nos. 1463, 1465, 1466, 1469, 1511, 1689, and 1691) were statistically better than PEG 540, and seven TSPs (ICD Nos. 1690, 1692, 1467, 1536, 1509, 1623, and 1621) were statistically indistinguishable from PEG 540. None of the TSPs tested was worse than PEG 540 against a VX challenge (see page B-9).

Only three TSPs (ICD Nos. 1463, 1465, and 1511) consistently ranked in the top five against all three organophosphonate agents used in the Phase 1 in vitro tests.

3.2 Phase 1, Initial Efficacy In Vivo

Of the 14 TSPs tested against TGD in the in vivo portion of Phase 1, five TSPs (ICD Nos. 1469, 1511, 1465, 1466, and 1692) were statistically better than PEG 540, four TSPs (ICD Nos. 1689, 1467, 1690, and 1691) were statistically indistinguishable from PEG 540, and five TSPs (ICD Nos. 1536, 1621, 1463, 1509, and 1623) were worse than PEG 540 (see page B-16). There were no significant ($p > 0.05$, two-sided) effects on RAs of wearing any TSP for 1 hr (see page B-17). There were significant ($p < 0.05$, two-sided) increases in RAs from 120 min to 24 hr after dosing for eight rabbits pretreated with ICD Nos. 1691 and 1689. Significant ($p < 0.05$, two-sided) decreases in RAs, indicating possible delayed penetration or retention, from 120 min to 24 hr after dosing were observed for the eight-rabbit replicates pretreated with ICD Nos. 1466 and 1467 (see page B-18). Correlation of in vitro and in vivo results against TGD challenges are presented in Section 3.5 of this report.

Against an HD challenge, 11 TSPs (ICD Nos. 1465, 1689, 1469, 1511, 1691, 1466, 1536, 1467, 1463, 1690, and 1692) were statistically better than PEG 540, one TSP (ICD No. 1621) was equivalent to PEG 540, and two TSPs (ICD Nos. 1509 and 1623) were worse than PEG 540 (see page B-26).

Only three TSPs (ICD Nos. 1465, 1469, and 1511) consistently ranked in the top five against both challenge agents used in the Phase 1 in vivo tests. In all Phase 1 in vitro and in vivo test rankings, only two TSPs (ICD Nos. 1465 and 1511) consistently ranked in the top five.

3.3 Phase 2, Functional Testing

All work accomplished under Phase 2 is presented in a letter report dated 9 January 1991, and attached as Appendix C, "Letter Report on Phase 2, Functional Testing." That letter report compares results of stressing selected TSPs with either water or time of wear in Phase 2 with results of the same, unstressed TSPs from Phase 1. Initially, five TSPs (ICD Nos. 1465, 1466, 1469, 1511, and 1536) were recommended by USAMRICD investigators for Phase 2 testing based on their respective Phase 1 performance. However, the USAMRDC directive to limit Phase 2 testing to ICD Nos. 1465, 1511, and 1536 was issued after testing of all five TSPs had commenced at the MREF. Thus, water stress testing was nearly completed for all five TSPs, but time stress testing was completed for the three TSPs specified by USAMRICD.

See page C-5 for a summary of functional testing against an HD challenge. The effect of increasing the TSP wear time from 1 hr to 4 hr when challenged by HD was significantly ($p < 0.05$, two-sided) detrimental to ICD No. 1536. ICD Nos. 1465 and 1511 were not significantly affected. The effect of water stressing the TSP before HD application was also significantly detrimental to ICD No. 1536. ICD Nos. 1465 and 1469 were unaffected by water, but the efficacies of ICD Nos. 1466 and 1511 against HD were statistically (but probably not biologically) significantly improved by water stressing.

See pages C-9 and C-10 for a summary of functional testing against a TGD challenge. ICD No. 1536 efficacy against TGD was enhanced by time stressing as indicated by blood samples taken within 1 hr after dosing. Time stressing ICD No. 1465 was somewhat detrimental, but ICD No. 1511 was unaffected. Water stressing was somewhat detrimental to ICD No. 1465 but

somewhat beneficial to ICD Nos. 1466 and 1469. In particular, water stressing ICD No. 1466 apparently prevented a 4-to-24-hr decrease in AChE RAs in eight rabbits tested. The lack of consistent significance across blood sample times for these effects should lead the reader to view such isolated significance with some skepticism. ICD Nos. 1511 and 1536 were unaffected by water.

In summary, the efficacy of ICD No. 1536 was adversely affected by time and water stressing against a HD challenge, but was apparently enhanced by time stressing against a TGD challenge. Data indicated other, isolated, statistically significant effects related to TSP stressing that were probably not biologically significant, however.

3.4 Phase 3, Advanced Efficacy

All work accomplished under Phase 3 is presented in a letter report dated 24 June 1991, and attached as Appendix D, "Letter Report on Phase 3, Advanced Efficacy Testing."

Against a GD challenge, ICD Nos. 1511 and 1465 were significantly ($p < 0.05$, Tukey's multiple comparisons test) better than PEG 540, and ICD No. 1536 was indistinguishable from PEG 540 (see page D-10). There were no significant ($p > 0.05$, two-sided) effects on RAs of wearing any TSP for 1 hr (see page D-11). There were significant ($p < 0.05$, two-sided) increases in RAs from 120 min to 24 hr after dosing for six rabbits pretreated with ICD No. 1511 (see page D-12).

Against a VX challenge, ICD Nos. 1511 and 1536 were significantly better than PEG 540, and ICD No. 1465 was indistinguishable from PEG 540 (see page D-18). There were no significant effects on RAs of wearing any TSP for 1 hr (see page D-19). There was a significant decrease in RAs, indicating delayed penetration, from 120 min to 24 hr after dosing for seven rabbits pretreated with ICD No. 1536 (see page D-20).

Against HL challenge, ICD Nos. 1511 and 1465 were significantly better than PEG 540, and ICD No. 1536 was indistinguishable from PEG 540 (see page D-26).

Notably, ICD No. 1511 protected better than the other TSPs tested against penetration by all three agents used in Phase 3.

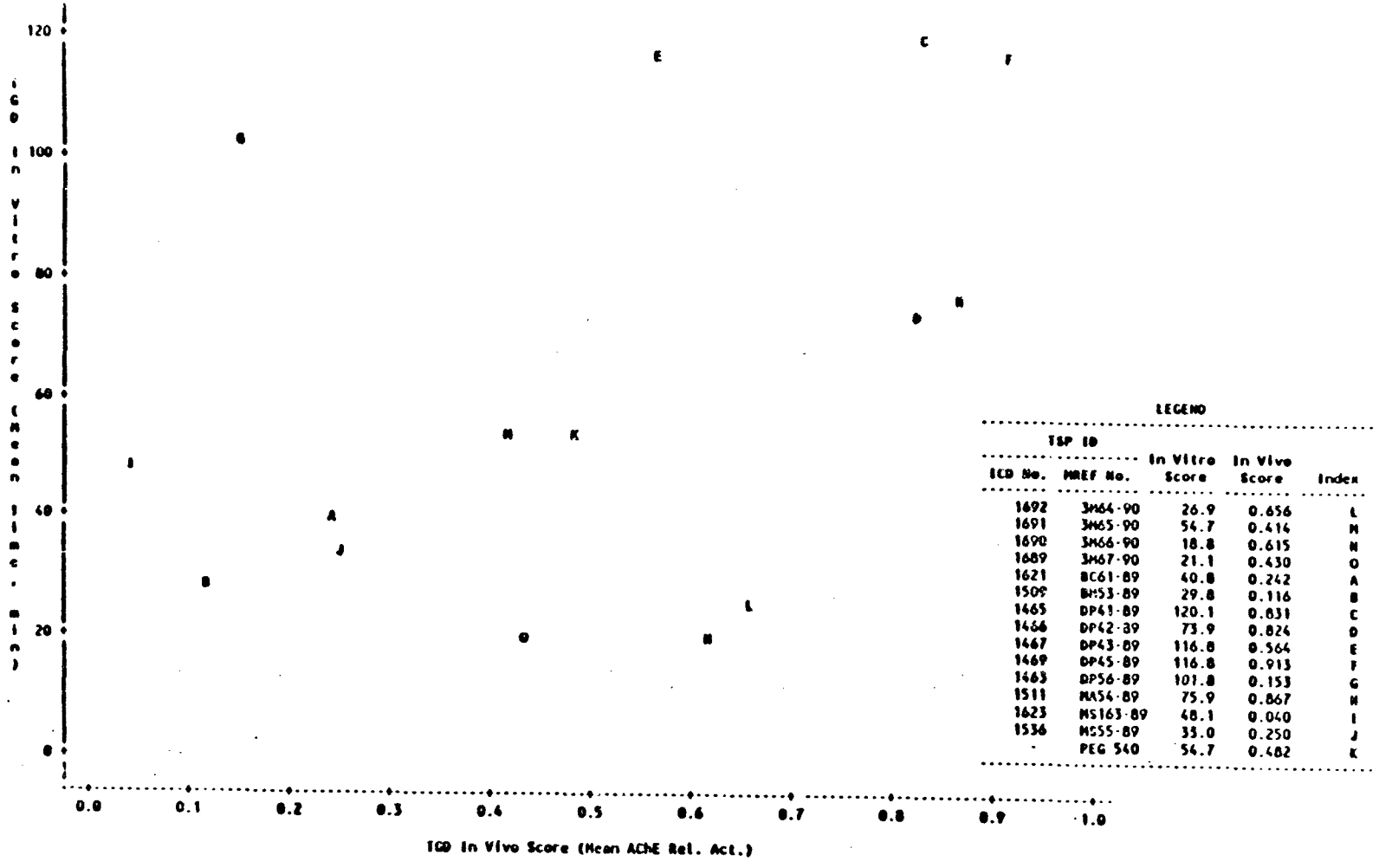
3.5 Correlations of TSP Performance by In Vitro Versus In Vivo Tests

Figures 1, 2, and 3 are correlation plots of TSP efficacy against organophosphonate penetration by the *in vitro* end point (y axis) versus the *in vivo* end point (x axis) for TGD, GD, and VX, respectively. On each plot, TSPs with index symbols at the top were highly efficacious against agent penetration and delayed for up to 2 hr the inhibition of eel AChE in receptor fluid in the penetration cell. TSPs with index symbols toward the right side of each figure were highly efficacious against agent penetration and protected rabbits from RBC AChE inhibition relatively well. Thus, a good positive correlation between *in vitro* and *in vivo* methods would be implied by an arrangement of index symbols in a straight line with a positive slope. A significantly ($p < 0.05$) positive correlation would imply that the *in vitro* method was a reliable predictor of testing the TSPs *in vivo*.

In Figure 1, the index symbols appear in three categories. Most of the TSPs (indexed by I, B, A, J, M, K, D, and H) form a straight, narrow band with a positive slope. TSPs indexed by G, E, C, and F are all fluorinated greases from DuPont and performed much better in the *in vitro* model than on the rabbit backs. The third group (indexed by O, L, and N) were all fluorochemicals from 3M Corporation and were the three worst TSPs against TGD in the *in vitro* model. For unknown reasons, they performed better than expected against TGD in the *in vivo* model. The correlation coefficient (R) for all 15 points was 0.4388 and was nearly significant at the 10 percent level ($p = 0.1018$). Apparently, the protective efficacies of some TSPs are strongly influenced by factors, e.g., TSP/skin interaction and skin surface temperature and moisture, not present in the *in vitro* penetration models using Silastic or other synthetic, non-biologic membranes. We propose, therefore, that substituting skin or living skin equivalent for the lower layer of synthetic membrane in the *in vitro* model may greatly improve the correlation between the *in vitro* and *in vivo* test results.

In Figure 2, the index symbols indicate a good positive correlation ($R = 0.9488$, $p = 0.0512$) between performance results from the *in vitro* and *in vivo* models for TSP efficacy against GD. Although the data on hand indicate a statistical relationship, more TSPs would have to be tested before the *in vitro* model can be said to be predictive of *in vivo* results. That is,

FIGURE 1. TASK 89-03 PHASE 1 TGD IN VITRO RESULTS CORRELATED WITH PHASE 1 TGD IN VIVO RESULTS



LEGEND

TSP ID		In Vitro	In Vivo	
ECD No.	MREF No.	Score	Score	Index
1692	3M64-90	26.9	0.656	L
1691	3M65-90	54.7	0.414	M
1690	3M66-90	18.8	0.615	N
1689	3M67-90	21.1	0.430	O
1621	BC61-89	40.8	0.242	A
1509	BH53-89	29.8	0.116	B
1465	DP41-89	120.1	0.831	C
1466	DP42-89	73.9	0.824	D
1467	DP43-89	116.8	0.564	E
1469	DP45-89	116.8	0.913	F
1463	DP56-89	101.8	0.153	G
1511	MA56-89	75.9	0.867	H
1623	MS163-89	48.1	0.040	I
1536	MS55-89	33.0	0.250	J
-	PEG 540	54.7	0.482	K

FIGURE 2. TASK 89-03 PHASE 1 GD IN VITRO RESULTS CORRELATED WITH PHASE 3 GD IN VIVO RESULTS

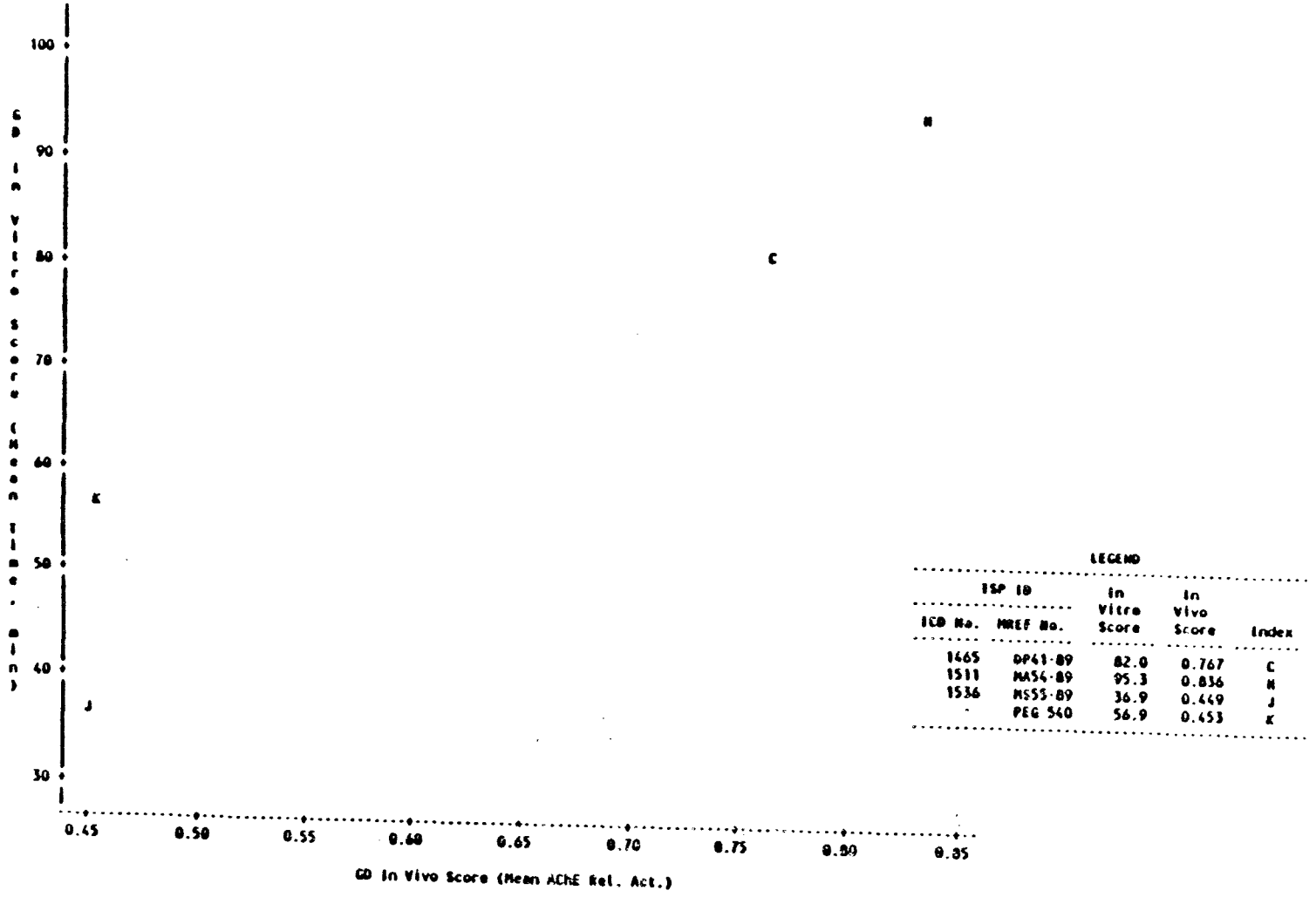
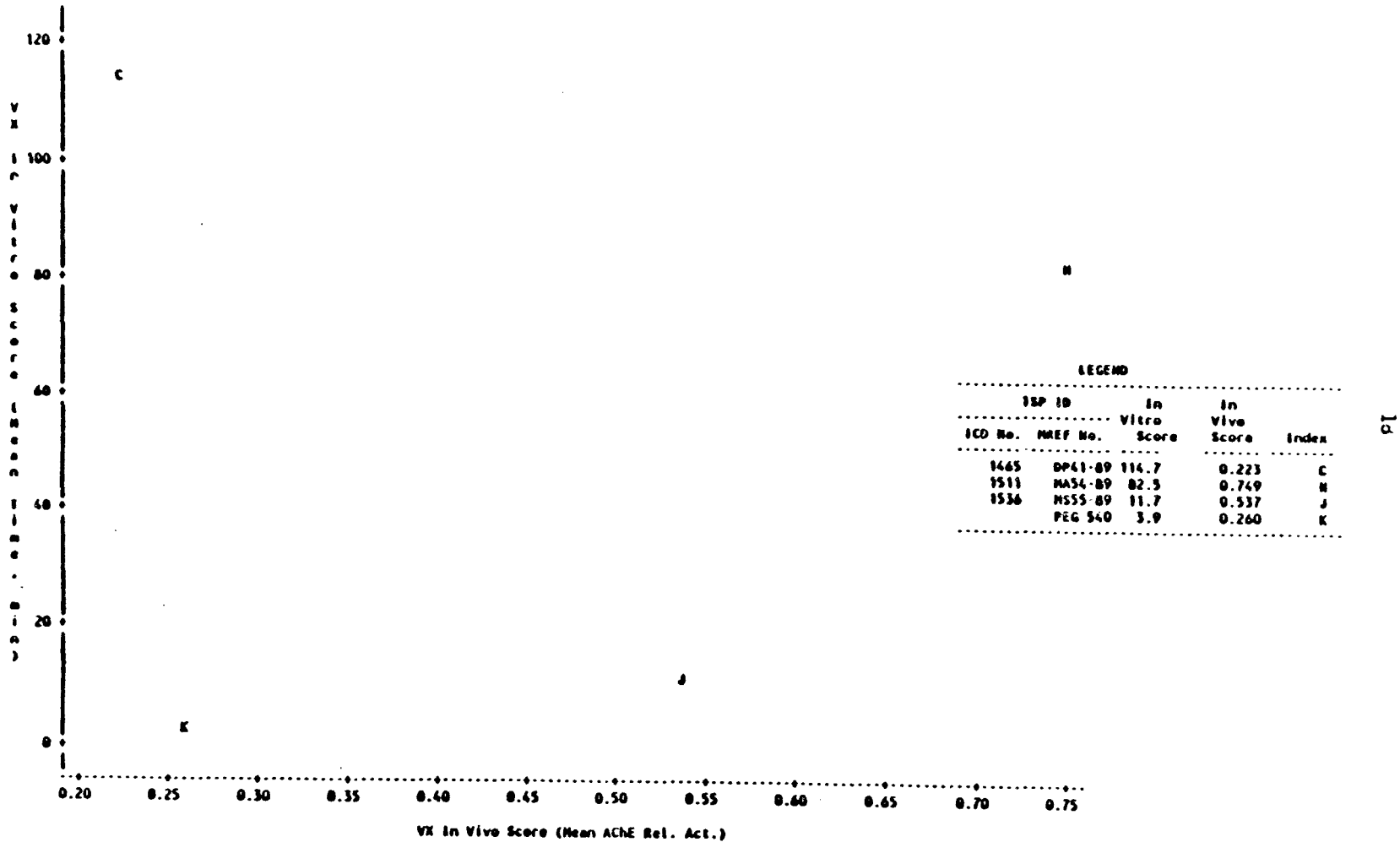


FIGURE 3. TASK 89-03 PHASE 1 VX IN VITRO RESULTS CORRELATED WITH PHASE 3 VX IN VIVO RESULTS



more points are needed to confirm the association between model results for GD challenge. Notably in Figure 2, ICD No. 1465 (index symbol "C") was not superior to ICD No. 1511 (index symbol "H") as in the TGD *in vivo* model, but fell in line with the other TSPs.

No association between test models was evident for VX, as shown in Figure 3 ($R = 0.0137$, $p = 0.9863$). Notably, ICD No. 1465 (index symbol "C") performed far better in the *in vitro* model than on rabbit backs. We have no explanation for why this material performed worse *in vivo* than what was predicted by the *in vitro* results. ICD No. 1465 may form a relatively impervious film when rubbed on rabbit epidermis that cannot be duplicated by applying it on Durapore membrane. Again, the paucity of data on hand preclude the making of any strong conclusions regarding the lack of association between the two models.

3.6 Special Reports

During the course of deployment of U.S. troops in Operations Desert Shield and Desert Storm, the MREF was tasked by USAMRICD and USAMRDC to perform five special studies, using *in vivo* Phase I methods, to determine the efficacy of several different manufacturer's lots of ICD No. 1536 (hereafter referred to as Multishield®) against a topical HD threat. The thrust of these studies was to demonstrate the efficacy of Multishield® against HD and obtain FDA approval for immediate fielding in the Middle East. Letter reports on these special studies were sent to USAMRDC in rapid succession in January and February 1991, and are included with this report as Appendices D, E, F, G, and H.

The original lot of Multishield®, Lot 5256, was shown in Phase I *in vivo* work to be statistically superior to PEG 540 against HD, with mean Scores of 0.188 and 0.509, respectively. The objective of the *first* special study was to determine whether a second manufacturer's lot of Multishield®, Lot 11JAN91BH, was more efficacious than PEG 540 against HD. Assessment of the second lot (mean Score = 0.530) showed it to be statistically ($p > 0.05$, two-sided) indistinguishable from PEG 540 (mean Score = 0.509, current and historical data combined). Obviously, there was a substantial difference between the efficacies of the two lots of Multishield®, attributable to either

formulation, preparation/packaging methods, or age-related factors. An examination of historical PEG 540 data indicated no evidence of drift in the test model between the periods of testing the two Multishield® lots. These results are reported in Appendix E, entitled, "The Efficacy of Lot 11JAN91BH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge."

The objective of the second special study was to compare Lot 11JAN91BH of Multishield® against no pretreatment to demonstrate its absolute efficacy at non-standard exposure times, i.e., 5, 30, and 60 min. Contralaterally paired (by rabbit) t tests of LARs demonstrated significant ($p < 0.05$, two-sided) efficacy at 5 and 30 min, but not at 60 min after dosing HD. The paired difference between mean Scores (0.250 for Multishield®, Lot 11JAN91BH, and 0.377 for no pretreatment) was significant, thus demonstrating absolute protective efficacy across that range of exposure periods. These results are reported in Appendix F, entitled "The Efficacy of Lot 11JAN91BH of the Topical Skin Protectant, ICD No. 1536, Relative to No Protectant, Against a Sulfur Mustard Challenge."

In the third special study, a third lot of Multishield®, Lot 17JAN91B, was tested against no pretreatment, to demonstrate absolute efficacy, and against PEG 540, to demonstrate relative efficacy. Standard protocol exposure times of 1, 2, and 4 hr were used. PEG 540 data included historical as well as current LARs and Scores. The tests indicated no absolute efficacy ($p > 0.05$) for Lot 17JAN91B and relative efficacy at 4 hr only. Mean Scores indicated no differences among Lot 17JAN91B, PEG 540, and no pretreatment. In short, there was no protection afforded against HD by wearing either Lot 17JAN91B or PEG 540. These results are reported in Appendix G, entitled "The Efficacy of Lot 17JAN91BH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge."

Results of the first three special studies indicated a dramatic difference between Lot 5256 and later lots of Multishield®. This generated speculation that the difference was due to a drift in the MREF Task 89-03 *in vivo* test model since the original testing of Multishield® Lot 5256 in February, March, and April 1990. An inspection of PEG 540 historical control data revealed no apparent drifts in the model. The fourth special study was performed to ascertain whether the efficacy of Multishield Lot 5256 could be

redemonstrated. Contrasts between the original and current test data for Lot 5256, PEG 540, and no pretreatment indicated this ranking in order of decreasing efficacy against HD:

Original Test, Lot 5256 = Current Test, Lot 5256 > PEG 540 > no pretreatment

This ranking was true for mean LARs at 1-, 2-, and 4-hr exposures and for mean Scores. Thus, the efficacy of Multishield® Lot 5256 was confirmed as unchanged and statistically ($p < 0.05$) superior to PEG 540. Apparently, the loss of efficacy in later lots of Multishield® was not due to a change in the test model, but rather to formulation, preparation/packaging methods, or age-related factors. These results are reported in Appendix H, entitled "The Efficacy of Lot 5256 of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge."

The objective of the fifth and last special study in Task 89-03 was to compare a fourth lot of Multishield®, Lot 03JAN91AH, to Lots 5256 and 11JAN91BH, and to PEG 540. Multiple comparisons were made between data obtained for these lots from previous special studies and current results to test for inter-study consistency. Comparisons were also made among data from the current study only. Inter-study contrasts for Lot 5256 indicated no significant ($p > 0.05$) differences, but Lot 11JAN91BH apparently improved (mean Score = 0.266) relative to its previous testing one month earlier in mid-January 1991 (mean Score = 0.530). This may be due to an aging effect. Comparisons among data from the fifth special study only indicated no distinction between Lots 03JAN91AH and 5256 for LARs at all exposure periods and for the mean Scores. Both were better than Lot 11JAN91BH at 1- and 2-hr exposures and for mean Scores, in spite of its possible age-related improvements. All three lots of Multishield® were statistically the same at the 4-hr exposure and were better than PEG 540 in all comparisons. These results are reported in Appendix I, entitled "The Efficacy of Lots 5256, 11JAN91BH, and 03JAN91AH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge."

4.0 CONCLUSIONS

Methods described in MREF Protocol 58 were used to screen 14 topical skin protectants (TSPs) in multiple-phase testing against a variety of organophosphonate and vesicant CSM. Stringent process quality control methods were employed to guarantee consistency in each of the test models across time. Of the 14 TSPs tested in Phase 1 against GD, TGD, and VX in an in vitro TSP penetration model and against TGD and HD on rabbit backs, five TSPs were identified as offering significantly improved protection relative to PEG 540. At the direction of USAMRDC, only three TSPs were fully tested in the functional efficacy and advanced efficacy phases. These were identified as ICD No. 1465 (Krytox fluorinated grease from Dupont), ICD No. 1511 (Fomblin perfluorinated grease from Montefluos Grappo Ausimont), and ICD No. 1536 (Multishield from Interpro, Inc). A summary of test results from all phases is presented in Table 3.

Plots, of TSP performance in an in vitro penetration cell model used in Phase 1 with the performance of TSPs applied on rabbit backs, indicated good correlation between the methods for GD and TGD challenges. Substitution of natural skin or living skin equivalent for the lower layer of synthetic membrane in the in vitro model may provide the interactions needed for TSPs to perform against CSM challenges as they would on rabbit backs. More work is needed in this area to develop an accurate, inexpensive alternative to in vivo testing. In addition, the in vitro model should be adapted for detecting the penetration of HD through candidate TSPs by substituting a suitable enzyme for eel AChE in the receptor fluid.

Testing related to rapid fielding of a suitable TSP for Operations Desert Shield and Desert Storm indicated ICD No. 1536 to be a somewhat unpredictable formulation. The original lot, 5256, was efficacious against HD. However a second lot, 11JAN91BH, exhibited absolute efficacy at 5- and 30-min HD exposures, but not at a 60-min exposure and was not better than PEG 540. A third lot, 17JAN91B, exhibited no efficacy, probably due to manufacturing and/or packaging variables (e.g., formulation temperature). A retest of Lot 5256 revalidated the MREF test model and re-established the efficacy of that lot of ICD No. 1536. Approximately one month after the first testing of Lot 11JAN91BH, a retest indicated significant improvement due to

material aging. A fourth lot, 03JAN91BH, was shown to be as efficacious as Lot 5256. This was a good indication that packaging and age of the product had a lot to do with its efficacy. Such instability and temperature-related efficacy were deemed undesirable for a TSP to be fielded in a desert climate.

TABLE 3. SUMMARY OF TOPICAL SKIN PROTECTANT SCREEN RESULTS

		Phase 1: Initial Efficacy					Phase 2: Functional Efficacy				Phase 3: Advanced Efficacy		
		In Vitro			In Vivo		Water Stress		Time Stress		In Vivo		
ICD No.	MREF No.	GD	TGD	VX	HD	TGD	TGD	HD	TGD	HD	GD	VX	HL
1463	DP56-89	+	+	+	+	-							
1465	DP41-89	0	+	+	+	+	d	n	d	n	+	0	+
1466	DP42-89	0	0	+	+	+	n	e					
1467	DP43-89	0	+	0	+	0							
1469	DP45-89	0	+	+	+	+	e	n					
1509	BM53-89	0	0	0	-	-							
1511	MA54-89	+	0	+	+	+	n	e	n	n	+	+	+
1536	MS55-89	0	0	0	+	-	n	d	e	d	0	+	0
1621	BC61-89	0	0	0	0	-							
1623	MS163-89	-	0	0	-								
1689	3M66-90	+	-	+	+	0							
1690	3M67-90	0	-	0	+	0							
1691	3M65-90	+	0	+	+	0							
1692	3M64-90	0	0	0	+	+							

+ = better than PEG 540
0 = same as PEG 540
- = worse than PEG 540
e = enhancement by stress
n = no effect of stress
d = detriment by stress

5.0 RECORD ARCHIVES

Records pertaining to the conduct of Task 89-03 are contained in Battelle laboratory record books and are archived at the MREF. These are shown in the following table by task phase to facilitate referencing.

TABLE 4. BATTELLE LABORATORY RECORD BOOKS USED IN MREF TASK 89-03

Phase 1, Initial Efficacy		Phase 2		Phase 3
In Vitro	In Vivo	Functional Testing	Advanced Efficacy	Special Studies
187	183	201	216	220
189	185	202	217	222
190	186	213		
192	188	214		
194		215		
197				
200				
203				
205				

All original data, as well as the original final report, will be maintained at the MREF until forwarded to USAMRDC at the conclusion of the project.

6.0 ACKNOWLEDGEMENTS

The name, role in the study, and highest academic degrees of the principal contributors in this study are presented in the following list:

Name	Title	Degree
Dr. Garrett S. Dill	MREF Manager	D.V.M.
Dr. David W. Hobson	Research Leader/ Study Director	Ph.D.
Mr. Thomas H. Snider	Study Coordinator	B.S.

APPENDIX A

MREF Protocol 58

Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HD/L

Study performed by Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201

1. MREF Manager: Garrett S. Dill, D.V.M.

2. Study Director: David W. Hobson, Ph.D.

3. Study Veterinarian: Peter L. Jepsen, D.V.M.

4. Sponsor: U.S. Army Medical Research and Development Command

5. COR: MAJ James R. Stewart, USAMRICD

6. Objective: To determine, using both in vitro and in vivo test procedures, the relative effectiveness of new candidate topical protectants in minimizing or preventing the toxic effects of several chemical surety materiels (CSMs) following topical exposure.

7. Experimental Design: The complete testing of each new candidate topical protectant (CTP) involves performance of several test procedures conducted in three phases, (1) initial efficacy, (2) functional testing, and (3) advanced efficacy. Summarized datasets for tests performed under each phase are provided to the sponsor at the completion of each phase and are used for selection of candidates to be tested in the next sequential phase. The CSMs to be used in these tests include GD, thickened GD (TGD), VX, HD, and HD/L. Specific guidance for the handling, storage and testing of each CTP is provided by the sponsor for each compound submitted. A diagram showing the relationship between each of the phases and data reporting scheme for the evaluation of CTPs is provided in attachment A.

   The initial efficacy phase is conducted in two parts, (a) an in vitro part, and (b) an in vivo part. The in vitro part is composed of penetration tests with VX, GD and TGD which are performed as described in MREF SOPs 89-61 and 89-65. The in vivo part is performed using New Zealand White rabbits as the percutaneous exposure model and with TGD and HD as challenge agents. The purpose of this phase is to determine where a given CTP is to be ranked for initial efficacy relative to other CTPs using HD or TGD as challenge agents. HD and TGD were selected for use in these tests because both present substantial cutaneous hazards and represent the two broad classes of chemical agents (i.e., vesicants and

nerve agents) for which topical protectants should be efficacious unless otherwise specified, a proprietary mixture of polyethylene glycols with average molecular weight of 540 (PEG 540) is used as a topical protectant control in all testing.

The functional testing phase is designed to determine the relative performance of CTPs against a challenge of either TGD or HD following stressing with either water or time of wear. PEG 540 is used as the control topical protectant for each test.

The advanced efficacy phase is conducted using GD, VX and HD/L as challenge agents. The test procedures used are identical to those used in the initial efficacy phase. The purpose of this phase is to determine the relative efficacy of each CTP against a broader spectrum of possible challenge agents. PEG 540 is also used to control each test.

As mentioned above, the procedures used to conduct the in vitro penetration tests required in the initial efficacy phase are given in MREF SOPs 89-61 and 89-65. The endpoint used to measure CTP efficacy for each nerve agent certified surety materiel (CSM) is inhibition of erythrocyte acetylcholinesterase activity (AChE), and the endpoint used for vesicant CSM challenges is dermal lesion area. Although in most cases, it is anticipated that both in vitro and in vivo test results will be submitted to the sponsor following completion of phase one testing, in vitro test results may be used, at the sponsor's discretion, to eliminate CTPs found during in vitro testing to be particularly unsatisfactory from further in vivo testing. Details describing the conduct of the specific in vivo test within each phase of the evaluation are provided below.

A. In vivo Test System

Albino rabbits were chosen for use in the in vivo portion of this study on the basis of the existing historical data base for this species, experience of the MREF staff in their care and handling, and because the rabbit provides an application area for CTPs which is suitable for challenges with neat CSM.

(1) Animals - Specific pathogen free (SPF) New Zealand White (albino) male rabbits, 8 per replicate group, three replicate groups per control or CTP per test.
Suppliers: Mohican Valley Rabbitry or Hazelton Laboratory Animals.

(2) Initial Weight - 2.0 to 4.0 kilograms.

(3) Quarantine - Rabbits are held in isolation and observed for clinical illness for at least 7 days prior to study initiation.

Quarantine may be performed at Battelle's King Avenue animal facility or at the MREF.

(4) Acclimation - All animals are held at the MREF at least 24 hr prior to study initiation.

(5) Selection - Animals selected after a minimum 7-day quarantine period are in good physical condition. Rabbits are then selected for study on the basis of proper weight and hair growth cycle stage. Selected rabbits are randomly assigned to weight-homogenized treatment groups for use on study.

(6) Animal Identification - Ear tag or tattoo; positive identification is required for each animal upon admission to quarantine. Cage cards, at a minimum, give animal number, sex, supplier and date of receipt for each rabbit.

(7) Housing - Animals are housed individually in stainless steel, slotted cages equipped with automatic watering systems.

(8) Lighting - Fluorescent lighting, light/dark cycle is 12 hr each per day.

(9) Temperature - Maintained at 21C (± 3C).

(10) Humidity - Maintained at 50 percent (± 10 percent).

(11) Diet - Purina Certified Rabbit Chow pellets are available at all times. No contaminants are known to be present in the feed which would interfere or affect the results of the study.

(12) Water Supply - Water is supplied from the public water system and given ad libitum. No contaminants are known to be present in the water which would affect the results of the study.

(13) Laboratory Animal Welfare Practices - Battelle's Animal Resources Facilities have been registered with the U.S. Department of Agriculture (USDA) as a rEsearch Facility (Number 31-21) since August 14, 1967, and are periodically inspected in accordance with the provisions of the Federal Animal Welfare Act. In addition, animals for use in research are obtained only from laboratory animal suppliers duly licensed by the USDA. Battelle's statement of assurance regarding the Department of Health and Human Services policy on humane care of laboratory animals was accepted by the Office of Protection from Research Risks, National Institutes of Health, on August 27, 1973. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the Care and Use of

Laboratory Animals" (DHHS Publication NO (NIH) 85-23) and/or in the regulations and standards are promulgated by the Agricultural Research Service, USDA, pursuant to the Laboratory Animals Welfare Act of August 24, 1966 as amended (P.L. 89-544 and P.L. 91-579).

(14) Accreditation - On January 31, 1978, Battelle Memorial Institute received full accreditation of its animal-care program and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. The MREF is a part of the Facilities granted full accreditation.

(15) Animal Care During Test - All animals are held in the MREF hood system on tie-down boards from the time of CSM dosing until decontamination up to 4 hr later. If required by the test, survivors are removed from the tie-down boards and are placed in stainless steel stanchions in the hood for up to 24 hr after CSM application. Upon completion of the study, all surviving rabbits are euthanized by intravenous injection of T-61 euthanasia solution and are disposed of by incineration after proof-of-decontamination (POD).

B. Test Groups

(1) Size - Each of the in vivo screening tests is performed using replicate groups of 8 animals per CTP or control material. Group matching is based on homogenous group weight and sex.

(2) Number - One replicate group of animals is used per control or CTP on each day of dosing, with a data collection rate of one satisfactory (as defined in Section K for each test type) dataset per day per material over three days of testing being required to complete each screening test (minimum, N=24 animals per CTP or control per test).

C. Test Materials

(1) Control material. PEG 540 obtained from Union Carbide Corporation is used as the control material in all CTP tests. Alternative control materials may be substituted for PEG 540 upon request by the Sponsor.

(2) CTPs are supplied by the sponsor. It is also the responsibility of the sponsor to ensure that appropriate identification (batch number, lot number, physical state, etc.), expiration date (if

available), safety and storage data are supplied for each CTP received by the MREF.

(3) GD, TGD, VX, HD and L are supplied by the USAMRDC. Purity, appropriate identification (batch number, lot number, state), and stability data are supplied by the USAMRDC. Purity and stability are confirmed periodically by Battelle. HD/L is prepared ate MREF for test use according to the procedures described in MREF SOP 88-38.

(4) Surety, security, and safety procedures for the use of each CSM are thoroughly outlined in facility plans, in personnel requirements for qualifications to work with agents, and in agent storage and use standard operating procedures. Specific procedures have been included in this document to ensure the safety of the personnel conducting this experiment.

C. Preparation of Animals for Testing

(1) Removal of Hair Coat - Rabbits acclimated in approved cages at the MREF for at least one day have their dorsal hair coat closely clipped from withers to rump with care to avoid skin damage using an Oster Model A-2 animal clipper with Number 40 blade, or equivalent, at least 24 hr prior to intended use.

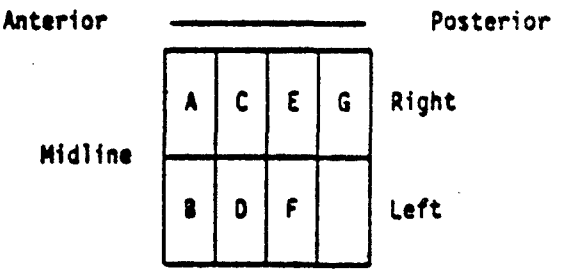
(2) Anesthesia - Rabbits are given 5.0 mg/kg (20 mg/ml) xylazine and 35.0 mg/kg (100 mg/ml) ketamine by intramuscular injection prior to the marking of test sites and CTP application. The time of administration is recorded. Rabbits are then placed in prone position on holding boards, each leg being taped with 1-inch wide cloth tape to prevent movement. In tests where CSM dosing is to occur more than 2 hr after the marking of test sites and topical protectant application, the animals are taken off the tie-down boards after application of the topical protectant, are fitted with an Elizabethan collar and are placed back in a stainless steel cage to recover. Re-administration of anesthesia to these animals, followed by collar removal and the placement of each rabbit on a tie-down board is initiated approximately 30 min prior to CSM dosing. Full anesthetic boosters of the same dose are administered after initial anesthesia used for restraint prior to CSM dosing or as needed during each test.

(3) Marking Test Sites - Prior to marking test sites, rabbits are reclipped, if necessary, to prevent shielding of exposure sites by hair stubble. Each rabbit is then marked with a felt-tip pen to outline each exposure site. The marking of nerve agent

exposure sites and preparation for dosing differs from that of vesicant exposure sites as follows:

(a) Nerve Agent CSM Exposure Sites - Each animal is anesthetized and secured to a tie-down board as described in Section 7. C (2). A 24-gauge, 1 mL catheter is inserted into the central artery of each ear of the rabbit for serial blood collection. After insertion the catheter is taped or glued in place and is filled with heparinized saline. The dorsum of each rabbit to receive CSM with or without barrier is marked, using a felt-tip pen, with a circle to mark the test site. The circle is 3.81 cm in diameter if a rubber "O" ring is to be applied to contain the dose (non-occlusive exposure), or 2.7 cm in diameter if a domed silicone rubber chamber is applied (occlusive or semi-occlusive exposures). If not specified, the default procedure is to use the rubber "O" ring.

(b) Vesicant CSM Exposure Sites - After each animal is anesthetized and secured to a tie-down board as described in Section 7. C (2), an application/dosing site grid as shown below is applied to the dorsum of each animal with a felt-tipped pen. Each site within the grid measures 5 cm from the midline by 2.5 cm wide. Care is taken to assure that the inside measurements of each dose site meet the required 2.5 cm x 5 cm dimensions.



D. Application of Topical Protectants

(1) Nerve Agent Exposure Sites -

(a) An aliquot, calculated to provide a uniform application depth of the control or candidate topical protectant is applied to the test site using a 1 mL disposable syringe (no needle). The standard uniform application depth is 0.1 mm

(application rate = 0.01 ml/cm2), but other application depths may be used depending on the requirements of the study. The volume of topical protectant required to produce an estimated application depth of 0.1 mm if the "O" ring procedure is used is 0.11 ml. If the domed silicone rubber chamber is used, the volume corresponding with a 0.1 mm application depth is 0.06 ml.

(b) A stainless steel spatula or glass rod is used to spread the material evenly about the exposure area. Care is taken such that little, if any, of the material from the exposure area is removed during the application process. Spreading is performed both with and against the direction of hair stubble growth. The time of application is recorded upon completion of spreading.

(c) A 3.5-cm inside diameter "O" ring or 3.3-cm outside diameter domed silastic chamber with an open top (dependent on the test site selection in Section 7.C.(3) is centered over the test site, and cemented onto the skin by applying a bead of cyanoacrylate adhesive around the surface of the ring or chamber in skin contact. If a completely occlusive application is required, a closed-top silastic chamber is cemented over the test site after nerve agent application in an approved CSM hood.

(d) Each topical protectant material is allowed to remain on the exposure area at least 1-hr before CSM application, unless otherwise specified by the test.

(2) Vesicant Agent Exposure Sites -

(a) Topical protectants are applied to sites A-F on the dorsum of each rabbit. Two topical protectants may be tested per rabbit, one applied to sites A,C and E and the other applied to sites B,D and F. Site G receives no application and serves as a non-protected control site.

(b) Each CTP is applied to eight rabbits per day of testing. The control topical protectant is also applied to eight rabbits per test day. A maximum of 24 rabbits (1 control topical protectant and 5 CTPs) may be exposed per test day.

(c) Each topical protectant material, control or CTP, is applied at a calculated uniform depth of 0.1 mm (application rate = 0.01 ml/cm2) to standardize application conditions. A 1-mL

disposable syringe (no needle) is used to deliver 0.13 mL of the topical protectant to each 2.5 cm x 5 cm exposure area. The application depth may vary, depending on the test requirements.

(d) A spatula is used to uniformly spread each topical protectant material about the exposure area, up to the grid marks on each side of the exposure area, to obtain a smooth and even coating. Care is taken to minimize any loss of material from the exposure area. The time of application is recorded.

(e) A space is maintained between each of the contiguous exposure areas, the width of the marker pen, where there is no protective coating applied. This boundary helps to maintain exposure site integrity throughout the study.

(f) Each topical protectant material is allowed to remain on the exposure area at least 1 hr before CSM application. Longer time periods may be specified by the specific test requirements.

E. Endpoint Measurement Procedures

(1) Nerve Agent Assays - The endpoint used to quantify topical protectant effectiveness is percent AChE inhibition relative to pre-exposure baseline values over a test-specified time period. Packed erythrocyte AChE activities are determined as described in MREF SOP-88-49 from heparinized blood samples of approximately 1.0 ml. Basically, the procedure uses an autoanalyzer with a photometer to quantitate the timed production of colored enzymatic reaction product (5-thio-2-nitrobenzoic acid) resulting from reactions of substrate and other test agents with AChE in the sample. Control of the assay is achieved by simultaneous analysis of AChE samples of known activity.

Because packed erythrocyte AChE measurements are occasionally subject to error due to sample hemolysis, criteria for sample acceptability must be used in order to identify and control this source of error. Until suitable procedures to detect sample hemolysis are established, selective procedures for the reanalysis of suspect samples are to be followed to determine the acceptability of AChE values for each sample. If indicated, reanalysis of a sample is conducted by repeating each of the steps involved in sample preparation and analysis as described in MREF SOP 88-49. Due to sample stability considerations, each samples must be analyzed, or reanalyzed, within 24 hr of its

collection time. As indicated in MREF SOP-88-46, samples are to be stored on ice or refrigerated prior to analysis. Values from samples found to be unacceptable upon reanalysis are eliminated from all subsequent computations involving the dataset.

(a) Baseline Samples - The acceptability of individual baseline sample values is determined using historical rabbit baseline sample values (expressed in Units/ml packed erythrocytes) as a guide. All baseline samples with AChE values outside ± 3 standard deviation units from the historical mean are reanalyzed. The new value is then used as the baseline value if, upon reanalysis, the new value falls within the limits of acceptability. If the reanalysis value falls outside the limits of acceptability, then the baseline value for the animal is suspect and all subsequent data obtained from that animal are also suspect and are not used in topical protectant efficacy computations. The historical baseline dataset is updated using all values found to be acceptable following the completion of each test.

(b) Nerve Agent Inhibited Samples - Determining the acceptability of the data from these samples is, in this study, somewhat complicated by the variable effects of the topical protectant material on the rate and extent of AChE inhibition. Nevertheless, the acceptability of data from each sample can be determined because, unless there is no change from the baseline value (indicating complete topical protectant effectiveness), a pattern of progressive AChE inhibition is the expected pattern as nerve agent exposure time increases. Sample hemolysis, if it occurs, usually results in causes abnormally high AChE values and such values are unacceptable for use in the evaluation of CTP efficacy. Based on previous experience, the influence of sample hemolysis on test results can be significantly reduced by incorporation of a few steps to determine sample acceptability during each test. The steps in determining sample acceptability are: (1) all nerve agent-inhibited samples within a timed series for each rabbit are analyzed and the data are tabulated as a percentage of baseline AChE activity on an individual rabbit basis, (2) all samples with baseline percentage AChE values in excess of 10 percent of the previous value in the time series are reanalyzed, (3) reanalysis results which return a value in excess of 10 percent of the previous value in the time series are considered suspect and are not used in topical protectant efficacy computations, (4) all other reanalysis results are used to replace the previous result in the efficacy

computations and the previous result is recorded as "suspect sample hemolysis" in the study records.

(2) Vesicant Assays - The endpoint used to quantify the effectiveness of topical protectants against vesicant CSMs is exposure time dependent lesion area (measured in mm^2 and expressed as a percentage of a 24 hr, unprotected control site lesion area). The times of exposure to vesicant CSMs are specified by each individual test. Lesion area evaluations are performed as described below:

(a) Dye Injection - At 20-24 hr after exposure, each animal is given a 2.0-mL intramuscular injection, in each thigh, of a 3 percent suspension of Trypan blue dye in saline. The dye requires at least two hours to translocate throughout the damaged vessels of the exposure areas. The dye forms a dark blue marking of the lesion against the contrasting pale blue of adjacent normal skin. A pink halo may extend 2-4 mm wider than the blue zone, which presumably is indicative of active hyperemia.

(b) Anesthesia - Approximately 2-4 hr after administration of the dye and just prior to taking lesion measurements, the test animals are anesthetized with 35.0 mg/kg of ketamine and 5.0 mg/kg of xylazine.

(c) Lesion Area Determination - After anesthesia, at approximately 24 hr after exposure, the lesion at each exposure site is measured with the use of a plastic metric ruler. Measurements of the length and width (longest axis in each direction) of each affected area are obtained. These measurements are recorded and lesion areas are calculated based on an elliptical area formula. Representative lesions may be recorded photographically, if required by the Sponsor.

(d) After lesion area determination is completed, each animal is euthanized by lethal injection of an approved euthanasia solution (e.g. T-61).

F. Application of CSM Challenge to Animals

(1) During CSM dosing and throughout the exposure period for each test, rabbits are positioned inside exposure CSM hoods to maintain air flow of approximately 100 linear ft/min, anterior to posterior, over the rabbit. Besides personnel safety, this positioning helps to eliminate the possibility of CSM inhalation exposures affecting the AChE results.

(2) Nerve Agent Assays

(a) The application of agent to each rabbit is made at a test-specified time following application of the topical protectant material. All safety procedures for the percutaneous application of G and V agents provided in MREF SOP-83-1 are observed.

(b) GD, TGD or VX is applied to the marked area of backs of restrained rabbits with a sharp-tipped needle to provide a point-source, air-dropped delivery without smearing or accidental penetration of the topical protectant.

(c) Delivery of TGD or GD is performed using a calibrated micrometer syringe. VX is applied using a Hamilton microliter syringe.

(d) Doses applied are based on the results of preliminary AChE determinations for each test scenario and are related to historical unprotected LD_{50} values for each agent applied percutaneously to unprotected rabbits. The doses are those estimated to produce approximately 80 ± 10 percent AChE inhibition relative to pre-exposure baseline values for rabbits protected with the control material following a 2 hr exposure to each CSM. Doses may be adjusted as needed by logarithmic increments of the historical LD_{50} value in order to accommodate new control materials or the particular needs of the sponsor. For the 0.1 mm standard application depth, using PEG 540 as the control topical protectant, the doses estimated for TGD, GD and VX application are 3.35, 1.35 and 0.3 mg/kg or 1.0, 1.0 and 10.0 times the LD_{50} value, respectively.

(3) Vesicant Assays -

(a) The application of HD or HD/L to each animal is made at a test-specified time after topical protectant application. All safety procedures given in MREF SOP-83-2 are observed during this operation.

(b) A 1 µL volume of HD or HD/L is applied to the center of each exposure site, using a Hamilton 7001N syringe with a sharp-tipped, positive displacement needle to provide a point source, air-dropped delivery without smearing or accidental penetration of the barrier. If a droplet of vesicant CSM remains on the end of the needle, the needle may be brought down close to the barrier (but without coming in contact

with the topical protectant) so as to "wick" off the droplet onto the topical protectant.

(c) The challenge dose of vesicant CSM remains on the appropriate topical protectant coated exposure area for either 1, 2, or 4 hr after application, unless otherwise specified by the test procedure.

(d) HD or HD/L is applied to the 24-hr control site (site G) in the same manner as all other exposure areas.

(e) The seven exposure areas are dosed in alphabetical order (A→G) with a 30-sec interval between each dose.

G. Decontamination

(1) Nerve Agents -

(a) Exposed animals are kept within an approved CSM hood until they are decontaminated and euthanized.

(b) At the end of the CSM exposure period specified for each test, the protective coating and agent is removed by wiping the area with a dry paper swab, which is discarded into a beaker containing either 10 percent sodium hydroxide (NaOH) for TGD or GD, or 5 percent sodium hypochlorite (NaOCl) for VX.

(c) The exposure area is then thoroughly rubbed for at least 5 sec with a gauze pad saturated with 10 percent NaOH or 5 percent NaOCl (see G.1.b). This pad is also discarded into the beaker containing 10 percent NaOH or 5 percent NaOCl after use.

(d) Step 7.G. (1) c is repeated once.

(e) The exposure area is then rinsed twice with deionized water to remove any traces of NaOH or NaOCl.

(f) The animals are then placed in metal stanchions and held overnight, if specified by the test.

(g) The rabbits are euthanized at the conclusion of the test and the carcasses are placed in double plastic bags. The bags are then sealed and are removed from the hood for proof-of-decontamination and disposal by incineration.

(2) Vesicants -

(a) Study-Specific Decontamination - This decontamination procedure is performed to remove excess vesicant and to decontaminate the exposure areas at specified exposure times. It is not the final decontamination procedure used for removal of animals from the hood system.

(i) Each exposure site is decontaminated at the test-specified time period after vesicant application (e.g. A, B at 1 hr; C, D at 2 hr; E, F at 4 hr and the control site, G, is not decontaminated).

(ii) Each exposure site is carefully dabbed with a 4 in. x 4 in. gauze pad attached to a tongue depressor to remove excess CSM from the surface of the topical protectant. Care is exercised so as to cause minimal disturbance to the surface of the topical protectant.

(iii) Each exposure site is next decontaminated using a plain swab (absorbent padding wrapped and attached to a tongue depressor) which contains 3.0 mL of a 5.0 percent NaOCl solution. The exposure area is gently contacted with each side of the decontaminant pad for 10 sec. This procedure is then repeated.

(ii) The exposure area is then contacted two individual times, 10 sec per side, with plain swabs containing 3.0 mL of distilled H_2O. This is done to eliminate or minimize irritation caused by the NaOCl solution.

(v) After decontamination, the animal is removed from the tie-down board and placed in a metal stanchion or standard caging for the remainder of the study period.

(b) General Decontamination - All animals receive an additional decontamination just prior to the animal's removal from the hood system.

(i) Lesion area measurement is completed and the animals are euthanized.

(ii) After euthanasia, the whole back of each animal carcass is decontaminated with a soaking wipe of 5 percent NaOCl

(iii) Carcasses are placed in double plastic bags which are sealed, removed from the hood for proof-of-decontamination and disposal by incineration.

H. Initial Efficacy Test Procedures

(1) In Vitro Tests - These tests are performed as described in MREF SOPs 89-61 and 89-65. The results from these tests may be used to eliminate, at the sponsor's discretion, the further in vivo testing of a CTP compound or formulation.

(2) In Vivo Nerve Agent Test - This test is performed using TGD only.

(a) Rabbits are prepared for study as described in Section 7. C for nerve agent tests. Eight rabbits per CTP or control topical protectant are used per day. Each day of testing is considered a test replicate.

(b) A baseline AChE sample is collected from each animal 5 min prior to topical protectant application.

(c) Topical protectants are applied to each rabbit as described in Section 7. D. (1) for nerve agent tests.

(d) Serial blood samples are collected from each rabbit at 5 min prior to TGD application (-5 min) and at 120 min post-TGD application. Blood samples are analyzed for AChE activity as described in Section 7.E. (1).

(e) Following a 1-hr period after topical protectant application, application of a TGD challenge is made to each rabbit as described in Section 7. F. (2) for nerve agent tests.

(f) The test is successfully completed following replication, three times on three different days according to the statistical criteria described in Section 7.K. for successful nerve agent tests.

(g) The rabbits from at least one test replicate are decontaminated as described in Section 7.G. (*) items (b) through (e), removed from their tie-down boards, placed in a metal stanchion and are allowed to recover from anesthesia. These animals are held for 24 hr post-TGD application in the hood and are given water ad libitum. At the end of the 24-hr period, a blood sample is collected and analyzed for AChE activity as described in Section 7. E. (1).

(h) For all other test replicates, the completion of blood collection is at 120 min and each test animal is euthanized, decontaminated and disposed of as described in Section 7. G.

(3) In Vivo Vesicant Test - This test is performed using HD only.

(a) Rabbits are prepared for study as described in Section 7. C. for vesicant agent tests. Eight animals are used per CTP or control topical protectant on each test day. A test day is considered a test replicate. Three successful replicates, as described in Section 7. K. (2), are required to complete the test for each CTP.

(b) Control and CTP materials are applied to the designated exposure sites on the dorsum of each rabbit as described in Section 7. D. (2) for vesicants.

(c) Following a 1-hr wait period after topical protectant application, the HD challenge dose is applied to each exposure site as described in Section 7. F. (3) for vesicants.

(d) Decontamination of exposure sites is performed as described in Section 7.G. (2) for vesicants at the following specified exposure times for each application site:

Sites A and B = 1 hr
Sites C and D = 2 hr
Sites E and F = 4 hr

(e) Site G is a 24 hr non-protected, exposure control site which is decontaminated 24 hr following vesicant application as described in Section 7. G. (2).

(f) Following the 4 hr decontamination, each rabbit is removed from the tie-down board and is placed in a metal stanchion. The rabbits are then held for approximately 20 hr in the CSM hood and are given water ad libitum.

(g) Each rabbit is prepared and each application site is evaluated for vesicant-induced lesion area as described in Section 7. E. (2).

(h) Following lesion area determination, the animals are euthanized and are prepared for proof of decontamination and disposal as described in Section 7. G. (2). The lesion area data are compiled and statistically evaluated as described in Section 7. K. (3).

I. Functional Test Procedures

(1) Nerve Agent Challenge Following Water Stress - This test is conducted by washing a fixed application of each CTP compound with water and then challenging the "stressed" CTP with TGD.

(a) Preparation and number of rabbits used for the test is as described in Section 7. H. (2) a. for nerve agent tests.

(b) A baseline AChE sample is collected from each animal 5 min prior to topical protectant application.

(c) Topical protectants are applied to each animal as described in Section 7. D. (1).

(d) After application, each CTP is washed with a quantity of deionized water (22 ± 5 C) equal to 500 times the volume of the CTP applied. A graduated volumetric pipette is used to gravity deliver the deionized water.

(e) The control topical protectant is not washed.

(f) Following a 1-hr period after topical protectant application, application of a TGD challenge is made to each rabbit as described in Section 7. F. (2) for nerve agent tests.

(g) A TGD challenge (same dose used in the initial efficacy test) is applied as described in Section 7. F. (2) 1-hr after completion of the water stress for CTP compounds and 1.0 hr after application of the control topical protectant. This permits drying of the application site prior to TGD challenge.

(h) The test is successfully completed following replication, three times on three different days according to the statistical criteria described in Section 7. K. for successful nerve agent tests.

(i) The rabbits from at least one test replicate are decontaminated as described in Section 7.G. (1) items (b) through (e), removed from their tie-down boards, placed in a metal stanchion and are allowed to recover from anesthesia. These animals are held for 24 hr post-TGD application in the hood and are given water ad libitum. At the end of the 24-hr period, a blood sample is collected and analyzed for AChE activity as described in Section 7. E. (1).

(j) For all other test replicates, the completion of blood collection is at 120 min and each test animal is euthanized, decontaminated, and disposed of as described in Section 7. G.

(k) The AChE data are then used to evaluate the relative effectiveness of each CTP as described in Section 7 K. (2).

(2) Nerve Agent Challenge Following Time Stress - This test is performed by allowing the rabbit to wear a fixed CTP application for a specified period of time and then challenging the "stressed" CTP with TGD.

(a) Preparation and number of rabbits used for the test is as described in Section 7. H. (2) a. for nerve agent tests.

(b) A baseline AChE sample is collected from each animal 5 min prior to topical protectant application.

(c) Topical protectants are applied to each animal as described in Section 7. D. (1).

(d) After application, each CTP is worn for a specified time period to test its wearability characteristics against a TGD challenge. The time period for the test is specified by the sponsor for each CTP.

(e) The control topical protectant (e.g. PEG 540) is not worn for the specified time period, rather it is worn for a standardized period of 1 hr.

(f) Nerve Agent Challenge Following Water Stress - This test is conducted by washing a fixed application of each CTP compound with water and then challenging the "stressed" CTP with TGD.

(g) A TGD challenge (same dose used in the initial efficacy test) is applied as described in Section 7. F. (2) after completion of Sponsor specified time periods for CTP compounds and 1 hr after application of the control topical protectant. If unspecified, the time period used for testing CTP compounds is 4 hr.

(h) The test is successfully completed following replication, three times on three different days according to the

statistical criteria described in Section 7. K. for successful nerve agent tests.

(i) The rabbits from at least one test replicate are decontaminated as described in Section 7.G. (1) items (b) through (e), removed from their tie-down boards, placed in a metal stanchion and are allowed to recover from anesthesia. These animals are held for 24 hr post-TGD application in the hood and are given water ad libitum. At the end of the 24-hr period, a blood sample is collected and analyzed for AChE activity as described in Section 7. E. (1).

(j) For all test replicates, the completion of blood collection is at 120 min and each test animal is euthanized, decontaminated, and disposed of as described in Section 7. G.

(k) The AChE data are then used to evaluate the relative effectiveness of each CTP as described in Section 7. K. (2).

(3) Vesicant Challenge Following Water Stress - This test is similar to that described in Section 7. I. (1) for nerve agents except that the vesicant test format is employed.

(a) Rabbits are prepared for study as described in Section 7. C. for vesicant agent tests.

(b) Control and CTP materials are applied to the designated exposure sites on the dorsum of each rabbit as described in Section 7. D. (2) for vesicants. Sites E and F are not used in this test.

(c) After application, each CTP application site is washed with a quantity of deionized water (22 ± 5 C) equal to 500 times the volume of the CTP applied. A graduated volumetric pipette is used to deliver the deionized water.

(d) Control topical protectant application sites are not washed.

(e) A HD challenge (same dose used in the initial efficacy test) is applied to each application site 1 hr after completion of the water stress for CTP compounds and 1 hr after application of the control topical protectant. This permits drying of the application site prior to HD challenge.

(f) The HD challenge dose is applied to each exposure site as described in Section 7. F. (3) for vesicants.

(g) Decontamination of exposure sites is performed as described in Section 7. G. (2) for vesicants at the following specified HD exposure times for each application site:

Sites A and B = 1 hr
Sites C and D = 2 hr

(h) Site G is a 24-hr non-protected, exposure control site which is decontaminated 24 hr following vesicant application as described in Section 7. G. (2).

(i) Following the 2-hr decontamination, each rabbit is removed from the tie-down board and is placed in a metal stanchion. The rabbits are then held for approximately 20 hr in the CSM hood and are given water ad libitum.

(j) Each rabbit is prepared and each application site is evaluated for vesicant-induced lesion area as described in Section 7. E.(2).

(k) Following lesion area determination, the animals are euthanized, prepared for proof of decontamination and disposal as described in Section 7. G. (2). The lesion area data are compiled and statistically evaluated as described in Section 7. K. (3).

(4) Vesicant Challenge Following Time Stress - This test is similar to that described in Section 7. I. (2) for nerve agents except that the vesicant format is used.

(a) Rabbits are prepared for study as described in Section 7. C. for vesicant agent tests.

(b) Control and CTP materials are applied to the designated exposure sites on the dorsum of each rabbit as described in Section 7. D. (2) for vesicants. Sites E and F are not used in this test. Because inflammation is a time-related response, exposure to HD in this test must occur at the same relative time of CTP or control topical protectant wear for each rabbit. Thus, when two topical protectants are to be tested per rabbit (i.e. one applied on the A and C sites and one on the B and D sites) the intended time of wear (and hence, HD application) must be similar for both protectants.

(c) After application, each CTP is worn for a specified time period to test its wearability characteristics against an HD challenge. The time period for the test is specified by the sponsor for each CTP. If unspecified, the default time period is 4 hr.

(d) The control topical protectant (e.g. PEG 540) is not worn for the test-specified time period, rather it is worn for a standardized period of 1 hr. This would mean in many cases that a CTP cannot be tested concurrently using the same animal used for the control application. When this is the case, it is acceptable to use both sets of test sites (i.e. A and C, B and D) for control application and data collection.

(e) An HD challenge (same dose used in the initial efficacy test) is applied to each application site after completion of the specified time period for CTP compounds and 1-hr after application of the control topical protectant.

(f) The HD challenge dose is applied to each exposure site as described in Section 7. F. (3) for vesicants.

(g) Decontamination of exposure sites is performed as described in Section 7. G. (2) for vesicants at the following specified HD exposure times for each application site:

Sites A and B = 1 hr
Sites C and D = 2 hr

(h) Site G is a 24 hr non-protected, exposure control site which is decontaminated 24 hr following vesicant application as described in Section 7. G. (2).

(i) Following the 2-hr decontamination, each rabbit is removed from the tie-down board and is placed in a metal stanchion. The rabbits are then held for approximately 20 hr in the CSM hood and are given water ad libitum.

(j) Each rabbit is prepared and each application site is evaluated for vesicant-induced lesion area as described in Section 7. E. (2).

(k) Following lesion area determination, the animals are euthanized, prepared for proof of decontamination and disposal as described in Section 7. G. (2). The lesion area data are compiled and statistically evaluated as described in Section 7. K. (2).

J. Advanced Efficacy Test Procedures

(1) VX Challenge - This test is conducted exactly as described in Section 7. H. (2) for nerve agent initial efficacy tests except that VX is used as the challenge agent instead of TGD.

(2) GD Challenge - This test is conducted exactly as described in Section 7. H. (2) for nerve agent initial efficacy tests except that GD is used as the challenge agent instead of TGD.

(3) HD/L Challenge - This test is conducted exactly as described in Section 7. H. (3) for vesicant agent initial efficacy tests except that HD/L is used as the challenge agent instead of HD.

K. Statistical Methods:

(1) In Vitro Nerve Agent Tests -

Each elution time value associated with a receptor fluid sample is adjusted by the time lag incurred in eluting the volume of receptor fluid in the tubing between the diffusion cell and the fraction collector. Results of GD-inhibited samples were expressed as relative inhibition (RI) by the following transformation:

$$RI = \frac{A_c - A_s}{A_c} \times 100 \text{ percent}$$

where A_c and A_s are the AChE activities in the concurrent control and sample fractions, respectively. The parameters used to characterize the efficacy of a material as a protectant against GD penetration in each replicate are the times after dosing to three levels of RI, initially 25, 50, and 75 percent (T_{25}, T_{50}, and T_{75}, respectively). These times may be decreased in number to one or two for test replicates involving penetration so rapid that the times are statistically indistinguishable. Any candidate protectant that prevents penetration of applied agent to the extent that RI is less than 95 percent at 2 hr is automatically passed onto the next screening tier. Thus, elution samples are not collected beyond 2 hr after dosing.
Each replicate is characterized by regressing an appropriate model (eg, a fifth-order polynomial or alternatively a cumulative distribution function) on the RI data as a function of lag-adjusted collection times. T_{25}, T_{50}, and T_{75} are determined from the regression parameter values by Newton's method for finding roots of equations. Mean T_{25}, T_{50}, and T_{75} are calculated for the candidate protectant across replicate runs.

Mean T_{25}, T_{50}, and T_{75} for PEG 540 are control charted and pooled across all replicates for all candidate protectants. Replicates with all three times outside the control chart's 95 percent confidence limits are not pooled with data from the other replicates. Thus, rank ordering the candidate materials relative

to each other and PEG 540 is delayed until all testing is completed. Initial replicates are judged as acceptable or unacceptable based on pilot study data, but the pilot study data are discarded from the data set for the final analysis and rank ordering. Analysis of variance followed by Tukey's test for multiple, simultaneous comparisons (alpha = 0.05) is performed to indicate relative efficacies among the candidates and PEG 540. The rank order of candidates is based on a composite evaluation of mean T_{25}, T_{50}, and T_{75} for each candidate and PEG 540.

(2) In Vivo Nerve Agent Tests -

(a) Quality Control - Control of each nerve agent is accomplished for each test day by control charting current mean relative (baseline-normalized) activity levels in erythrocyte samples for the control topical protectant with historical levels. The mean relative activity level at each time point after nerve agent application is charted with historical values at the respective times using an acceptance range of ± 3 standard deviations from the historical mean. The test replicate is considered valid if the mean relative activity levels obtained at all three time points fall within ± 3 standard deviations of the corresponding historical means. Invalid replicates are repeated. The test is considered complete for each CTP when three valid replicates of the control are obtained. There must be at least six complete sets of AChE values, control and CTP, in each eight-animal dataset. All replicate data is added in sequential order to the historical dataset.

(b) Comparison of CTP Efficacy - This is performed on statistically-controlled data by statistical comparisons of mean percent AChE activity values obtained for each CTP obtained under comparable test conditions and blood sample collection times. The percent AChE activity means and standard deviations for each CTP tested under each test condition up to the reporting date are determined for each animal per CTP relative to its individual baseline AChE value and the means are ranked from highest to lowest at each sample collection time and in a composite fashion by the mean of summation values across all sample times (except the 24 hr values). The 24 hr percent AChE mean for each CTP is statistically compared (one-sided t-test, alpha =0.05) to the corresponding 4 hr mean to in order to detect whether or not a significant ($p \leq 0.05$) decrease in the mean percent AChE value occurred following decontamination. The ranked CTP means are then statistically compared (alpha = 0.05) using a multiple comparison test (i.e. Tukey's procedure). In addition, functional test results AChE means are

statistically compared (t-test, alpha = 0.05) to corresponding values obtained for each CTP during the conduct of the initial efficacy tests in order to estimate whether the efficacy of the CTP was significantly affected ($p \leq 0.05$) by either the time or water functional challenge.

(3) Vesicant Tests -

(a) Statistical Control - Statistical control of each vesicant test is accomplished by statistical comparison of historical reference values for lesion areas from control topical protectants and the non-protected exposure sites, with corresponding values obtained from each replicate experiment in the test. Data from rabbits whose non-protected lesion area values fall ± 3 standard deviation outside the historical mean value for such exposures are not included as part of the test replicate. Mean lesion area values for the control topical protectant are statistically compared between the corresponding historical control value at each exposure time point after vesicant agent application and the mean values obtained from each replicate within the test to determine if the values are within ± 3 standard deviations of the corresponding historical control values. There must be at least six complete sets of usable lesion area values, control and CTP, in each eight-animal replicate dataset. The test replicate is considered statistically valid if the mean lesion area values obtained for all time points from the control topical protectant application sites in the replicate fall within ± 3 standard deviations of their corresponding historical values. All data from replicate datasets are added, in sequential order, to the corresponding sets of values within the historical control dataset. Invalid replicates are repeated. The test is considered complete for each CTP when three statistically valid replicates of the control are obtained.

(b) Comparison of CTP Efficacy - This is performed on statistically- controlled data by statistical comparisons of mean lesion area values obtained for each CTP obtained under comparable test conditions and exposure times. The lesion area means and standard deviations for each CTP treated site tested under each test condition up to the reporting date are ranked from lowest to highest at each exposure time and in a composite fashion by the addition of lesion area values across all exposure times. The means are then statistically compared (alpha = 0.05) using a multiple comparison test (i.e. Tukey's procedure). In addition, lesion area means from functional tests are statistically compared (t-test, alpha = 0.05) to corresponding values obtained for each CTP

during the conduct of the initial efficacy tests in order to indicate whether the efficacy of the CTP was significantly affected ($p \leq 0.05$) by either the time or water functional challenge.

9. Records to be Maintained:

   A. Compound inventory, specifications, and usage

   B. Dosage preparation and administration

   C. Animal receipt and quarantine records

   D. Animal data from all tests performed

   E. *In vitro* test data

   F. Decontamination results and disposal records

10. Reports:

   A. Letter Reports

      Each letter report contains a brief narrative description of the CTP test results obtained in each phase of testing. These are submitted to the COR within seven working days after the end of each phase.

   B. Final Report

      A final report is prepared and submitted within 30 days after completion of the task. It includes at least the following:

      (1) Signature page for key study individuals and their responsibilities.

      (2) Experimental design

      (3) *In vitro* and *in vivo* test data.

      (4) Test material description.

      (5) Application procedures.

      (6) Tabulation of *in vitro* and *in vivo* response data for each CTP tested.

      (7) Statistical methodology used.

(8) Discussion.

11. Approval Signatures:

David W. Hobson, Ph.D.
Study Director — Date 1/16/90

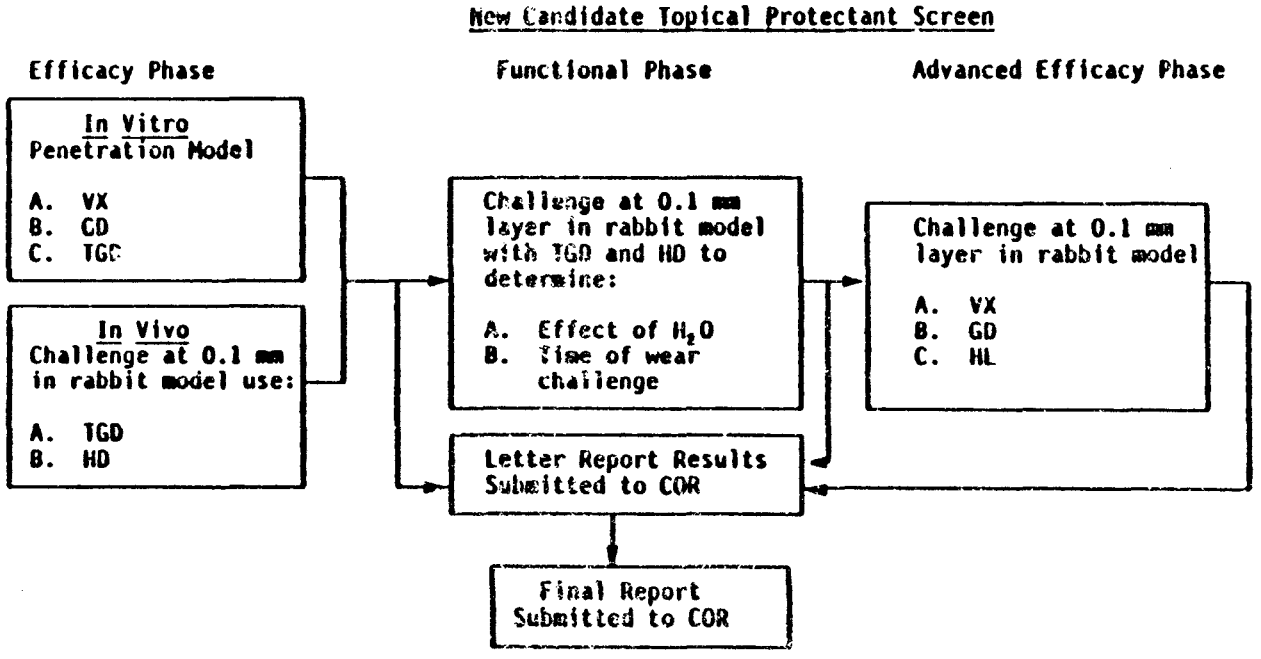
Garrett S. Dill, D.V.M.
Program Manager — Date 1/16/90

Peter L. Jensen, D.V.M.
Chief Veterinarian — Date 1/16/90

MAJ James R. Stewart Ph.D.
USAMRICD COR — Date 16 Jan 90

N/A - Non GLP Study
Quality Assurance Unit
Health and Environment Group — Date

N/A - Non GLP Study
Charles K. Burdick, Director
Total Quality Program
Health and Environment Group — Date


New Candidate Topical Protectant Screen
Efficacy Phase
Functional Phase
Advanced Efficacy Phase
In Vitro
Penetration Model
A. VX
B. GD
C. TGD
In Vivo
Challenge at 0.1 mm
in rabbit model use:
A. TGD
B. HD
Challenge at 0.1 mm
layer in rabbit model
with TGD and HD to
determine:
A. Effect of H_2O
B. Time of wear
challenge
Challenge at 0.1 mm
layer in rabbit model
A. VX
B. GD
C. HL
Letter Report Results
Submitted to COR
Final Report
Submitted to COR

Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HD/L

Protocol Amendment No. 1

Change: Replace Pages 9 and 10, Section 7.E.(1)(a) and (b) with the following:

(a) Baseline Samples - The acceptability of individual baseline (-65 min, pre-CTP application) sample values is determined using historical -65 min sample values (expressed in Units/mL packed rabbit erythrocytes) as a guide. All -65 min samples with AChE values outside ± 2 standard deviations from the historical mean are reanalyzed. The -65 min sample value becomes the average of all results within ± 3 standard deviations from the historical mean. If both values fall outside ± 3 standard deviations then the -65 min sample for the animal is suspect, as are all subsequent samples obtained from that animal, and none of the data are used in CTP efficacy computations. The historical data set is updated using all -65 min sample values found to be acceptable following the completion of each test.

(b) Nerve Agent Inhibited Samples - The acceptability of data from each nerve agent-inhibited sample can be determined because, unless there is no change from the baseline value (indicating complete topical protectant effectiveness), a pattern of progressive AChE inhibition is the expected pattern as nerve agent exposure time increases. Hemolyzed samples from the rabbit usually result in abnormally high AChE values that are unacceptable for use in the evaluation of CTP efficacy. Based on previous experience, the influence of sample hemolysis on test results can be significantly reduced by incorporation of following procedure to determine sample acceptability during each test.

An initial estimate of within-sample variability is obtained by measuring packed rabbit erythrocyte AChE activity levels from five preparations of the same sample and calculating a standard deviation. This is performed in multiple samples covering the range of anticipated activity levels. Within-sample variability is expressed as the standard deviation calculated as a function of activity levels determined by regression analysis. Three of these within-sample standard deviations is regarded as a tolerance limit term used in accepting test generated activity levels, as described below.

The steps in determining sample acceptability are:

(i) Each -5 min (just before agent dosing) sample activity value is compared with that rabbit's baseline value. If it is beyond the upper tolerance limit, i.e., the baseline plus the within-sample variability (three standard deviations) tolerance limit term, the -5 min sample is reanalyzed. If the second analysis level is less than that limit, it replaces the original analysis level. Otherwise, both data are omitted from the data set.

(ii) All nerve agent-inhibited samples within a timed series for each rabbit are analyzed and the data are tabulated as a percentage of baseline AChE activity on an individual rabbit basis. For animals moribund or dead as a result of agent intoxication, all subsequent AChE activity and baseline-normalized values for time periods following the death of that animal are recorded as zero and included in the statistical analysis.

(iii) All samples with AChE activity values in excess of the upper tolerance limit (the mean of the two most recent, accepted sample AChE activity levels for that rabbit plus the tolerance limit term defined above) are reanalyzed. If a second analysis level is greater than the tolerance limit, then both data are omitted from the data set.

Reason: Estimations of within-sample variability were made based on repeated sample analyses to replace the tolerance limit term (10 percent of the preceding activity level) arbitrarily assigned in the protocol. Experience has shown the 10 percent rule to be unnecessarily restrictive in determining acceptable data.

Impact on Study: Fewer sample AChE activity levels outside the upper tolerance limit, and decreased frequency of reanalysis.

Change: Page 22, Section 7.K.(2)(a).

There must be at least five acceptable AChE activity values at each time period, per control and CTP, in each eight-animal data set.

Reason: Power calculations have indicated that requiring six acceptable data points per set of eight animals instead of five data affords only marginal improvement (two or three percent in both directions from the PEG 540 mean relative activity) in detecting a significant difference between PEG 540 and a CTP at alpha = 0.05, beta = 0.10.

Impact on Study: The frequency of repeating study replicates necessitated by insufficient data will be decreased at minimal risk to the sensitivity of the design.

David W. Hobson, Ph.D.
Study Director

1/31/90
Date

MAJ James R. Stewart
USAMRICD COR

1/31/90
Date

Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HD/L

Protocol Amendment No. 2

Change: Page 1. Section 5.

MAJ James R. Stewart, D.V.M. is replaced with LTC Don W. Korte, Jr., Ph.D.

Reason: MAJ Stewart was replaced by LTC Korte as Contracting Officer's Representative.

Protocol Amendment No. 3

Change: Replace Section K.(3)(a) with the following:

(3) Vesicant Tests -

(a) Statistical Control - Statistical control of each vesicant test is accomplished by statistical comparison of historical reference values for lesion areas from control topical protectants and the non-protected exposure sites, with corresponding values obtained from each replicate experiment in the test. Data from rabbits whose non-protected lesion area values fall ± 3 standard deviation outside the historical mean value for such exposures are not included as part of the test replicate. Mean lesion area values for the control topical protectant are statistically compared between the corresponding historical control value at each exposure time point after vesicant agent application and the mean values obtained from each replicate within the test to determine if the values are within ± 3 standard deviations of the corresponding historical control values. There must be at least six complete sets of usable lesion area values, control and CTP, in each eight-animal replicate dataset. The test replicate is considered statistically valid if the mean lesion area values obtained for at least one time point from the control topical protectant application sites in the replicate fall within ± 3 standard deviations of their corresponding historical values. All data from replicate datasets are added, in sequential order, to the corresponding sets of values within the historical control dataset. Invalid replicates are repeated. The test is considered complete for each CTP when three statistically valid replicates of the control are obtained.

Reason: The current topical protectant control level is too stringent relative to the use of PEG 540 as the control topical protectant and the size of the existing PEG 540 historical dataset.

Impact on Study: There will be little or no change in the relative rankings for the topical protectants evaluated. This change eliminates the need to repeat tests unnecessarily in order to obtain a level of historical control which, for practical reasons, is too stringent for the use of PEG 540 as the control topical protectant.

David W. Hobson, Ph.D.
Study Director

3/20/90
Date

Don W. Korte, Jr.,
USAMRICD COR

20 mar 90
Date

Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HD/L

Protocol Amendment No. 3

Change: Replace Page 4, Section 7.8. (2) with the following:

(2) Number - One replicate group of animals is used per control on each day of dosing. For CTP replicates, the standard data collection requirement is one satisfactory data set (as defined in Section K for each test type) per day per material over at least three days of testing for test completion (ie, nominally N = 24 animals per CTP per test). As determined by the USAMRICD COR, very special circumstances may occasionally require the testing of three or more replicate groups of the same CTP on a single day. Under these circumstances, all subsequent references in this protocol to the number of test days and the number of animals to be treated with a given CTP per test day are superseded. CTP replicate groups of eight animals each are sequentially ordered, and the data are statistically treated as a though they were collected across multiple test days in all subsequent data handling operations required within the context of this protocol.

Reason: The above change allows for a decrease in the number of days required for testing a CTP from three to one in the event that the demand (e.g. data needed to support ongoing military operations) for the test results takes priority over the statistically preferred method.

Impact on Study: Possibly smaller within-day variances, but also possibly larger variances across days, in control data sets.

David W. Hobson
David W. Hobson, Ph.D.
Study Director

1/15/91
Date

Don W Korte
LTC Don W. Korte, Jr., COR
USAMRICD

15 Jan 91
Date

THS/cah

Evaluation of New Candidate Topical Protectants Using In Vitro and In Vivo Models to Determine Their Relative Effectiveness Against Thickened GD, GD, VX, HD and HD/L

Protocol Amendment No. 4

Change: Replace Page 15, Section 7.H. (3) (d) with the following:

(d) Decontamination of exposure sites is performed as describe Section 7.G. (2) for vesicants at the following exposure t for each application site, unless otherwise specified:

Sites A and B = 1 hr
Sites C and D = 2 hr
Sites E and F = 4 hr

Reason: This change allows the times to decontamination to vary with spe requirements, while retaining 1-, 2-, and 4-hr exposure periods nominal times for initial efficacy testing.

Impact on Study: Increases the utility of this protocol to allow speci studies.

David W. Hobson, Ph.D.
Study Director

1/17/91
Date

LTC Don W. Korte, Jr., COR
USAMRICD

17 Jan 91
Date

THS/cah

APPENDIX B

Letter Report on Phase 1, Initial Efficacy Testing, Dated 17 August, 199(

For Review and Approval

No. [illegible]
Internal Distr [illegible]

Name	Initials	Date
Originator DW Hobson	DWH	8-17-90
Concurrence		
Approval BG Maiden	Per fax	8-17-90

TS Snider
DW Hobson
BG Maiden
RMO
GS Dill/File

August 17, 1990

LTC Don W. Korte, Jr., MS, COR
Battelle Memorial Institute
505 King Ave., JM-3
Columbus, Ohio 43201-2693

Dear LTC Korte:

Contract DAMD17-89-C-9050
Task 89-03

The attached information provides a tabulated summary of the results from Ta 89-03, Phase 1 in vitro studies with GD, TGD, and VX and in vivo rabbit exposures to TGD and HD. The tables include all Phase 1 results from testin 14 candidate topical protectants (CTPs). The data summarized have been both quality controlled according to MREF Protocol 58 and reviewed by our Quality Assurance Unit at King Avenue.

The endpoint in the in vitro studies is the time required, after dosing 0.5 of agent on a synthetic membrane bilayer assembly enclosing a 0.1 mm thickne of each candidate topical protectant (CTP) and mounted in a penetration cell to achieve a prescribed fractional degree of inhibition (i.e., 0.25, 0.50, a 0.75 of the control cell activity) for eel acetylcholinesterase (AChE) activity in receptor fluid samples. Data reduction involved two steps; firs nonlinear regression parameters were estimated for the data obtained from ea cell by analysis of relative inhibition versus time using a cubic cumulative distribution function (Statistical Analysis System Nonlinear Regression procedure, or SAS NLIN) then, Newton's method for finding roots of equations was used to estimate the times to prescribed inhibition defined by the regression parameters. The "Score" parameter is the overall average value f all time estimates obtained for each of the three prescribed degrees of inhibition.

The in vitro results are provided as two tables. In the first table, the CTI are arranged by the test priority assigned by Dr. Hammond of the U.S. Army Medical Research Institute for Chemical Defense. In the second table, the CTPs are numerically ordered from apparent most to least effective based on the data. The latter identifies groups of CTPs having statistically indistinguishable means, as determined by analysis of variance using the least-squares means method (SAS General Linear Models, or GLM, procedure). Statistically grouped rankings of the CTPs are indicated for the "Score" parameter.

For TGD and HD tested in vivo, tables are attached which provide the following information:

- statistics on wear time periods from application of each CTP to agent dosing (there were no statistically significant, i.e., $P < 0.05$, effects due to wear time for either TGD or HD)
- statistics on the raw endpoint measured at each time period, i.e., red blood cell acetylcholinesterase (AChE) activity (U/mL) for TGD, and lesion area (mm^2) for HD
- statistics on the relative endpoint calculated at each time period, i.e., red blood cell AChE activity divided by the baseline value (%) for TGD, and lesion area divided by the unprotected control site lesion area (%) for HD
- statistics on the relative endpoint at each time period, ordered from apparent most to least effective CTP; these tables identify groups of CTPs having statistically indistinguishable means, determined by analysis of variance with the least-squares means method (Statistical Analysis System General Linear Models, or SAS GLM, procedure)
- statistics on the mean relative endpoint, expressed as a fraction and referred to as Score, ordered from apparent most to least effective CTP; this table identifies groups of CTPs having statistically indistinguishable Scores, determined by analysis of variance with the least-squares means method (SAS GLM procedure)
- for TGD only, statistics and paired t-tests to determine the effect of each CTP on rabbit AChE absolute activity from just before application to 1 hr later
- for TGD only, statistics and paired t-tests to determine whether rabbit AChE relative activity levels changed from 120 min to 24 hr after dosing.

Also included is a correlation plot showing the relative performance of the 14 CTPs by in vitro tests with that by in vivo tests for TGD. The points fall roughly into three groups. The majority of the 14 CTPs (indexed with I - B - A - J - M - K - D - H) define a fairly linear trend that indicates increasing in vitro efficacy with increasing in vivo efficacy. Points indexed by G - E - C - F define a line that indicates a greater in vitro efficacy than would have been expected by the in vivo results. If the in vitro procedure was used as a first level tier screen, these four CTPs would likely have passed into the in vivo tier, but their relative efficacy might not have been confirmed in the animal model. The group comprised of O - N - L were all 3M materials. We suspect their poor in vitro performance against TGD penetration may be the results of protective factors lacking in the synthetic membrane model, but present in the animal model.

Based on the combined test results for all 14 CTPs conducted to date, it appears that compounds DP41-89, DP42-89, DP45-89, MA54-89, and 3M66-90 offer significant protection with reasonable consistency across all tests.

If I can be of any further assistance, please call me at (614) 424-5259.

Sincerely,

David W. Hobson

David W. Hobson, Ph.D., D.A.B.T.
Associate Manager
Medical Research and Evaluation Facility

DWH/cah

Attachment

cc: COL Michael A. Dunn, Commander, USAMRICD
LTC George C. Southworth, MS, Deputy Commander, USAMRICD
MAJ James Romano, MS, RAD V, USAMRDC
Ms. Ellen Mackenzie, Chief, PCMB, USAMRICD
Benjamin G. Maiden, Ph.D.

NREF TASK 89-03, PHASE 1 IN VITRO ASSESSMENT OF CANDIDATE TOPICAL PROTECTANTS INDEXED BY TIME (min) AFTER DOSING CD TO SPECIFIC ACHE INHIBITION LEVELS IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Testing Priority	ICD No.	NREF No.		Relative Inhibition Level 0.25	0.50	0.75	Score*
0	-	PEG 540	N	131	133	133	134
			Mean	54.9	57.2	58.6	56.9
			SD**	30.5	31.2	31.5	30.9
1	1466	DP42-89	N	12	11	11	12
			Mean	47.9	53.7	54.1	50.3
			SD	15.5	13.7	15.2	17.2
2	1467	DP43-89	N	12	12	12	12
			Mean	61.6	64.9	67.6	64.7
			SD	12.8	14.6	15.9	14.6
3	1465	DP41-89	N	12	12	12	12
			Mean	76.9	82.4	86.6	82.0
			SD	17.9	20.0	21.5	19.8
4	1469	DP45-89	N	12	12	12	12
			Mean	70.5	75.7	80.1	75.5
			SD	16.1	18.1	20.1	18.0
5	1511	MA54-89	N	12	12	12	12
			Mean	90.9	95.5	99.5	95.3
			SD	23.9	23.0	23.1	23.1
6	1509	BM53-89	N	12	12	12	12
			Mean	32.5	33.7	34.7	33.6
			SD	16.9	17.5	18.0	17.5
7	1621	BC61-89	N	16	16	16	16
			Mean	36.7	38.6	40.1	38.5
			SD	6.9	7.2	7.5	7.2
8	1623	MS163-89	N	12	12	12	12
			Mean	27.6	28.3	29.0	28.3
			SD	6.7	7.2	7.5	7.1
9	1536	MS55-89	N	12	12	12	12
			Mean	35.5	36.8	38.2	36.9
			SD	5.7	5.6	5.8	5.7
10	1463	DP56-89	N	12	12	12	12
			Mean	114.8	115.2	115.5	115.2
			SD	28.8	28.5	28.3	28.5
11	1692	3M64-90	N	12	12	12	12
			Mean	58.5	62.2	64.9	61.9
			SD	29.6	29.8	30.4	29.9
12	1691	3M65-90	N	11	11	11	11
			Mean	86.9	90.7	94.3	90.6
			SD	30.7	29.6	28.4	29.4
13	1689	3M66-90	N	11	11	11	11
			Mean	117.2	118.8	120.0	118.7
			SD	12.6	9.8	8.4	10.0
14	1690	3M67-90	N	12	12	12	12
			Mean	41.3	44.4	45.8	43.8
			SD	9.8	9.3	9.7	9.2

* Score = Mean time to relative inhibition levels
** SD = Standard deviation

MREF TASK 89-03, PHASE 1 RANKING OF CANDIDATE TOPICAL PROTECTANTS INDEXED BY TIME (min) AFTER DOSING GD TO SPECIFIC AChE INHIBITION LEVELS IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Order of Means	ICD No.	MREF No.		0.25	0.50	0.75	Score*	Grouping (Means with the same letter are equivalent)
1	1689	3M66-90	N	11	11	11	11	
			Mean	117.2	118.8	120.0	118.7	A
			SD**	12.4	9.8	8.4	10.0	
2	1463	DP56-89	N	12	12	12	12	
			Mean	114.8	115.2	115.5	115.2	A
			SD	28.8	28.5	28.3	28.5	
3	1511	MA54-89	N	12	12	12	12	
			Mean	90.9	95.5	99.5	95.3	A B
			SD	23.9	23.0	23.1	23.1	
4	1691	3M65-90	N	11	11	11	11	
			Mean	86.9	90.7	94.3	90.6	A B
			SD	30.7	29.4	28.4	29.4	
5	1465	DP61-89	N	12	12	12	12	
			Mean	76.9	82.4	86.6	82.0	A B C
			SD	17.9	20.0	21.5	19.8	
6	1469	DP45-89	N	12	12	12	12	
			Mean	70.3	75.7	80.1	75.5	B C D
			SD	16.1	18.1	20.1	18.0	
7	1467	DP43-89	N	12	12	12	12	
			Mean	61.6	64.9	67.6	64.7	B C D E
			SD	12.8	14.6	15.9	14.4	
8	1692	3M64-90	N	12	12	12	12	
			Mean	58.5	62.2	64.9	61	B C D E F
			SD	29.6	29.8	30.4	29.9	
9	-	PEG 540	N	131	133	133	136	
			Mean	54.9	57.2	58.6	56.9	C D E
			SD	30.5	31.2	31.5	30.9	
10	1466	DP62-89	N	12	11	11	12	
			Mean	47.9	53.7	56.1	50.5	C D E F
			SD	15.5	13.7	15.2	17.2	
11	1690	3M67-90	N	12	12	12	12	
			Mean	41.3	44.4	45.8	43.8	D E F
			SD	9.8	9.3	9.7	9.2	
12	1621	BC61-89	N	16	16	16	16	
			Mean	36.7	38.6	40.1	38.5	E F
			SD	6.9	7.2	7.5	7.2	
13	1536	MS55-89	N	12	12	12	12	
			Mean	35.5	36.8	38.2	36.9	E F
			SD	5.7	5.6	5.8	5.7	
14	1509	BM53-89	N	12	12	12	12	
			Mean	32.5	33.7	34.7	33.6	E F
			SD	16.9	17.5	18.0	17.5	
15	1623	MS163-89	N	12	12	12	12	
			Mean	27.6	28.3	29.0	28.3	F
			SD	6.7	7.2	7.5	7.1	

* Score = Mean time to relative inhibition levels
** SD = Standard deviation

MREF TASK 89-03, PHASE 1
IN VITRO ASSESSMENT OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY TIME (min) AFTER DOSING TGD TO SPECIFIC AChE
INHIBITION LEVELS IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Testing Priority	ICD No.	MREF No.		Relative Inhibition Level 0.25	0.50	0.75	Score*
0	-	PEG 540	N	130	130	131	131
			Mean	52.6	55.1	56.8	54.7
			SD**	26.9	26.3	27.4	26.2
1	1466	DP42-89	N	12	12	12	12
			Mean	70.7	73.9	77.1	73.9
			SD	22.7	23.1	24.2	23.3
2	1467	DP43-89	N	12	12	12	12
			Mean	114.8	117.5	118.0	116.8
			SD	20.3	19.1	18.3	19.0
3	1465	DP41-89	N	12	12	12	12
			Mean	119.8	120.2	120.3	120.1
			SD	12.8	12.9	12.9	12.9
4	1469	DP45-89	N	12	12	12	12
			Mean	113.1	117.7	119.4	116.8
			SD	20.7	16.8	15.0	17.1
5	1511	MA54-89	N	12	12	12	12
			Mean	70.6	76.7	80.3	75.9
			SD	17.8	19.2	20.4	19.0
6	1509	BH53-89	N	12	12	12	12
			Mean	29.1	29.8	30.4	29.8
			SD	6.6	6.5	6.4	6.5
7	1621	BC61-89	N	12	12	12	12
			Mean	39.2	40.9	42.3	40.8
			SD	8.8	9.2	9.5	9.2
8	1623	MB163-89	N	12	12	12	12
			Mean	44.2	48.2	49.9	48.1
			SD	12.9	13.8	14.4	13.7
9	1536	MS35-89	N	12	12	12	12
			Mean	32.1	33.1	34.0	33.0
			SD	4.0	4.2	4.4	4.2
10	1463	DP56-89	N	12	12	12	12
			Mean	101.6	101.8	102.0	101.8
			SD	31.9	31.5	31.2	31.5
11	1692	3M64-90	N	12	12	12	12
			Mean	26.0	26.9	27.8	26.9
			SD	14.1	14.5	14.9	14.5
12	1691	3M65-90	N	11	12	12	12
			Mean	49.7	54.7	56.7	54.7
			SD	28.4	29.9	31.1	30.0
13	1689	3M66-90	N	9	9	9	9
			Mean	17.4	18.8	20.1	18.8
			SD	10.1	10.1	10.2	10.1
14	1690	3M67-90	N	9	10	12	12
			Mean	21.3	21.9	21.7	21.1
			SD	13.5	12.7	12.7	12.7

* Score = Mean time to relative inhibition levels
** SD = Standard deviation

USAMRICD TASK 89-03, PHASE 1
RANKING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY TIME (min) AFTER DOSING TGD TO SPECIFIC AChE
INHIBITION LEVELS IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Order of Means	ICD No.	MREF No.		Relative Inhibition Level 0.25	0.50	0.75	Score*	Grouping (Means with the same letter are equivalent)
1	1465	DP41-89	N	12	12	12	12	
			Mean	119.8	120.2	120.3	120.1	A
			SD**	12.8	12.9	12.9	12.9	
2	1467	DP43-89	N	12	12	12	12	
			Mean	114.8	117.5	118.0	116.8	A
			SD	20.3	19.1	18.3	19.0	
3	1469	DP45-89	N	12	12	12	12	
			Mean	113.1	117.7	119.4	116.8	A
			SD	20.7	16.8	15.0	17.1	
4	1463	DP54-89	N	12	12	12	12	
			Mean	101.6	101.8	102.0	101.8	A B
			SD	31.9	31.5	31.2	31.5	
5	1511	MA54-89	N	12	12	12	12	
			Mean	70.6	76.7	80.3	75.9	B C
			SD	17.8	19.2	20.4	19.0	
6	1466	DP42-89	N	12	12	12	12	
			Mean	70.7	73.9	77.1	73.9	B C
			SD	22.7	23.1	24.2	23.3	
7	1691	3M65-90	N	11	12	12	12	
			Mean	49.7	54.7	56.7	54.7	C D
			SD	28.4	29.9	31.1	30.0	
8	-	PEG 540	N	130	130	131	131	
			Mean	52.6	55.1	56.8	54.7	C D E
			SD	24.9	26.3	27.4	26.2	
9	1623	MS163-89	N	12	12	12	12	
			Mean	46.2	48.2	49.9	48.1	C D E F
			SD	13.9	13.8	14.4	13.7	
10	1621	BC61-89	N	12	12	12	12	
			Mean	39.2	40.9	42.3	40.8	D E F
			SD	8.8	9.2	9.5	9.2	
11	1536	MS55-89	N	12	12	12	12	
			Mean	32.1	33.1	34.0	33.0	D E F
			SD	4.0	4.2	4.4	4.2	
12	1509	BM53-89	N	12	12	12	12	
			Mean	29.1	29.8	30.4	29.8	E F
			SD	6.6	6.5	6.4	6.5	
13	1692	3M64-90	N	12	12	12	12	
			Mean	26.0	26.9	27.8	26.9	E F
			SD	14.1	14.5	14.9	14.5	
14	1690	3M67-90	N	9	10	12	12	
			Mean	21.3	21.9	21.7	21.1	F
			SD	13.5	12.7	12.7	12.7	
15	1689	3M66-90	N	9	9	9	9	
			Mean	17.4	18.8	20.1	18.8	F
			SD	10.1	10.1	10.2	10.1	

* Score = Mean time to relative inhibition levels
** SD = Standard deviation

MREF TASK 89-03, PHASE 1
IN VITRO ASSESSMENT OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY TIME (min) AFTER DOSING VX TO SPECIFIC AChE INHIBITION LEVELS
IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Testing Priority	ICD No.	MREF No.		Relative Inhibition Level 0.25	0.50	0.75	Score*
0	-	PEG 540	N	133	136	135	136
			Mean	3.0	3.8	4.8	3.9
			SD**	8.7	10.1	11.3	9.9
1	1466	DP42-89	N	12	12	12	12
			Mean	78.1	89.4	89.7	85.8
			SD	45.7	46.4	46.1	44.2
2	1467	DP43-89	N	11	12	12	12
			Mean	13.5	17.9	20.9	17.6
			SD	3.7	5.3	6.5	5.1
3	1465	DP41-89	N	12	12	12	12
			Mean	114.3	115.1	115.1	114.8
			SD	35.8	36.0	36.0	35.9
4	1469	DP45-89	N	9	11	11	12
			Mean	73.6	83.6	96.9	83.0
			SD	46.6	48.0	48.4	45.5
5	1511	MA54-89	N	10	10	9	12
			Mean	57.2	88.4	109.9	82.5
			SD	37.3	44.6	38.5	36.9
6	1509	BM53-89	N	12	12	12	12
			Mean	6.9	9.5	10.8	9.1
			SD	3.4	5.7	6.4	5.0
7	1621	BC61-89	N	12	12	12	12
			Mean	2.8	3.3	3.8	3.3
			SD	2.4	3.0	3.6	3.0
8	1623	MB163-89	N	12	12	12	12
			Mean	6.8	8.3	10.4	8.5
			SD	11.6	13.7	14.3	13.1
9	1536	MS55-	N	12	12	12	12
			Mean	5.6	11.8	17.9	11.7
			SD	4.0	15.5	18.8	11.8
10	1463	DP56-89	N	12	12	12	12
			Mean	125.3	125.7	125.7	125.5
			SD	1.0	0.5	0.5	0.4
11	1692	3M64-90	N	12	12	12	12
			Mean	15.1	18.3	20.3	17.9
			SD	14.1	16.3	17.2	15.8
12	1691	3M65-90	N	12	12	12	12
			Mean	66.7	76.1	81.0	74.6
			SD	37.0	38.4	35.5	36.5
13	1689	3M66-90	N	12	12	12	12
			Mean	70.4	78.3	86.0	77.6
			SD	38.3	39.3	39.7	38.8
14	1690	3M67-90	N	12	12	12	12
			Mean	18.1	19.9	21.6	19.9
			SD	7.6	8.3	8.8	8.2

* Score = Mean time to relative inhibition levels
** SD = Standard deviation

MREF TASK 89-03, PHASE 1
RANKING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY TIME (min) AFTER DOSING VX TO SPECIFIC ACHE
INHIBITION LEVELS IN RECEPTOR FLUID RELATIVE TO CONTROL CELL

Order of Means	ICD No.	MREF No.		Relative Inhibition Level 0.25	0.50	0.75	Score*	Grouping (Means with the same letter are eqivalent)
1	1463	DP56-89	N	12	12	12	12	
			MEAN	125.3	125.7	125.7	125.5	A
			SD	1.0	0.5	0.5	0.6	
2	1465	DP61-89	N	12	12	12	12	
			MEAN	114.3	115.1	115.1	114.8	A B
			SD	35.8	36.0	36.0	35.9	
3	1466	DP62-89	N	12	12	12	12	
			MEAN	78.1	89.4	89.7	85.8	B C
			SD	45.7	46.4	46.1	44.2	
4	1469	DP45-89	N	9	11	11	12	
			MEAN	73.6	83.6	94.9	83.0	C
			SD	46.6	48.0	48.4	45.5	
5	1511	MS54-89	N	10	10	9	12	
			MEAN	57.2	88.4	109.9	82.5	C
			SD	37.3	45.6	38.5	36.9	
6	1689	3M66-90	N	12	12	12	12	
			MEAN	70.4	79.3	84.0	77.6	C
			SD	38.3	39.3	39.7	38.8	
7	1691	3M65-90	N	12	12	12	12	
			MEAN	66.7	76.1	81.0	74.6	C
			SD	37.0	38.4	35.5	36.5	
8	1690	3M67-90	N	12	12	12	12	
			MEAN	18.1	19.9	21.6	19.9	D
			SD	7.6	8.3	8.8	8.2	
9	1692	3M64-90	N	12	12	12	12	
			MEAN	15.1	18.3	20.3	17.9	D
			SD	14.1	16.3	17.2	15.8	
10	1467	DP43-89	N	11	12	12	12	
			MEAN	13.5	17.9	20.9	17.6	D
			SD	3.7	5.3	6.5	5.1	
11	1536	MS55-89	N	12	12	12	12	
			MEAN	5.4	11.8	17.9	11.7	D
			SD	4.0	15.5	18.8	11.8	
12	1509	BM53-89	N	12	12	12	12	
			MEAN	6.9	9.5	10.8	9.1	D
			SD	3.4	5.7	6.4	5.0	
13	1623	MS163-89	N	12	12	12	12	
			MEAN	6.8	8.3	10.4	8.5	D
			SD	11.6	13.7	14.3	13.1	
14	-	PEG 540	N	133	136	136	136	
			MEAN	3.0	3.8	6.8	3.9	D
			SD	8.7	10.1	11.3	9.9	
15	1621	BC61-89	N	12	12	12	12	
			MEAN	2.8	3.3	3.8	3.3	D
			SD	2.4	3.0	3.6	3.0	

*Score = Mean time to relative inhibition levels
**SD = Standard deviation

TASK 89-43, PHASE 1, IN VIVO, TBD STATISTICS
FOR CANDIDATE TOPICAL PROTECTANT YEAR
TIME BETWEEN APPLICATION AND DOSING

	Candidate Topical Protectant Bear Time (min)				
	N	MEAN	SD*	MINIMUM	MAXIMUM
3684-00	24	68.1	8.3	60	81
3685-00	24	66.6	8.6	60	80
3686-00	24	61.3	1.8	60	66
3687-00	24	61.4	2.0	60	66
BC81-00	24	80.8	11.9	60	87
BB82-00	24	66.6	3.6	60	71
DP41-00	24	67.3	1.5	65	70
DP42-00	24	66.6	5.4	[illegible]	78
DP43-00	24	63.2	5.7	57	74
DP45-00	24	61.4	7.1	61	80
JF53-00	24	63.5	2.6	60	68
MA54-00	24	80.6	10.3	61	88
ME103-00	24	67.1	3.8	61	72
MEA5-00	24	77.8	7.5	67	87
PEE 540	178	64.8	4.1	60	73

* Standard deviation.

TASK 89-03, PHASE 1, IN VIVO, TGD
STATISTICS FOR ABSOLUTE RED BLOOD CELL
ACHE ACTIVITY (U/mL) BY TIME

Testing Priority	TGD No.	MREF No.		SAMPLE TIME RELATIVE TO DOSING TGD					
				-65 min	-5 min	30 min	60 min	120 min	24 hr
0	-	PEG 540	N	175	175	174	174	174	56
			Mean	1.96	1.96	1.31	0.88	0.62	0.59
			SD*	0.33	0.39	0.60	0.55	0.45	0.43
1	1466	DP42-89	N	24	24	24	24	21	8
			Mean	1.89	1.80	1.70	1.55	1.33	0.48
			SD	0.32	0.45	0.36	0.31	0.45	0.46
2	1467	DP43-89	N	24	23	23	24	23	8
			Mean	1.88	1.90	1.32	1.03	0.82	0.44
			SD	0.23	0.38	0.73	0.73	0.67	0.47
3	1465	DP41-89	N	24	24	24	24	24	8
			Mean	1.97	1.87	1.79	1.68	1.35	1.14
			SD	0.36	0.30	0.38	0.36	0.51	0.58
4	1469	DP45-89	N	24	24	24	24	24	8
			Mean	1.81	1.76	1.68	1.71	1.52	1.46
			SD	0.27	0.28	0.22	0.32	0.32	0.47
5	1511	MA54-89	N	24	24	24	24	24	8
			Mean	1.99	2.06	1.89	1.73	1.57	1.38
			SD	0.29	0.40	0.42	0.41	0.42	0.44
6	1509	BM53-89	N	24	24	24	24	24	8
			Mean	1.96	1.96	0.38	0.16	0.13	0.09
			SD	0.34	0.40	0.38	0.14	0.11	0.12
7	1621	BC61-89	N	24	24	24	24	24	8
			Mean	1.96	1.95	0.81	0.42	0.17	0.25
			SD	0.42	0.45	0.71	0.38	0.14	0.25
8	1623	MS163-89	N	24	24	24	24	24	8
			Mean	1.98	1.93	0.10	0.06	0.06	0.03
			SD	0.36	0.39	0.10	0.09	0.10	0.08
9	1536	MS55-89	N	24	24	24	24	24	8
			Mean	1.31	1.85	0.77	0.36	0.27	0.25
			SD	0.28	0.35	0.59	0.32	0.13	0.10
10	1463	DP56-89	N	24	24	24	24	24	8
			Mean	1.86	1.80	0.54	0.16	0.14	0.11
			SD	0.26	0.27	0.33	0.09	0.08	0.12
11	1692	3M66-90	N	24	24	24	24	24	8
			Mean	2.14	2.13	1.68	1.48	0.98	0.87
			SD	0.29	0.40	0.27	0.59	0.54	0.55
12	1691	3M65-90	N	23	23	23	22	23	8
			Mean	2.06	2.05	1.28	0.78	0.51	0.68
			SD	0.35	0.40	0.44	0.53	0.43	0.52
13	1689	3M66-90	N	24	22	24	23	24	8
			Mean	2.09	2.16	1.74	1.23	0.91	1.20
			SD	0.36	0.43	0.58	0.55	0.54	0.61
14	1690	3M67-90	N	24	22	24	24	24	8
			Mean	2.16	2.15	1.31	0.87	0.56	0.66
			SD	0.33	0.31	0.73	0.61	0.46	0.77

* Standard deviation

TASK 89-03, PHASE 1, IN VIVO, TGD
STATISTICS FOR RED RED BLOOD CELL
ACHE ACTIVITY (%) BY TIME

Testing Priority	ICD No.	MREF No.		SAMPLE TIME RELATIVE TO DOSING TGD				
				-5 min	30 min	60 min	120 min	24 hr
0	-	PEG 540	N	175	176	176	176	56
			Mean	100.8	67.4	45.8	32.6	30.4
			SD*	13.7	29.3	28.8	24.2	21.7
1	1466	DP42-89	N	24	24	24	21	8
			Mean	94.9	91.7	83.5	73.4	25.1
			SD	15.2	18.7	19.5	27.3	22.6
2	1467	DP43-89	N	23	23	24	23	8
			Mean	99.1	71.2	55.7	44.0	24.0
			SD	16.0	39.5	38.3	34.5	26.6
3	1465	DP41-89	N	24	24	24	24	8
			Mean	96.1	92.0	87.1	70.2	68.8
			SD	16.0	17.8	21.5	28.6	33.6
4	1469	DP45-89	N	24	24	24	24	8
			Mean	98.0	96.0	95.8	84.2	85.3
			SD	12.5	14.3	20.6	15.1	23.1
5	1511	AA54-89	N	24	24	24	24	8
			Mean	103.3	94.8	86.5	78.9	68.9
			SD	13.3	14.7	14.3	20.5	13.0
6	1509	BH53-89	N	24	24	24	24	8
			Mean	100.5	20.0	8.1	6.8	4.4
			SD	14.6	20.0	6.8	6.1	6.1
7	1621	BC61-89	N	24	24	24	24	8
			Mean	100.3	41.4	22.3	9.0	15.0
			SD	13.1	34.0	21.1	9.1	17.9
8	1623	MS163-89	N	24	24	24	24	8
			Mean	97.5	5.2	3.3	3.5	1.3
			SD	12.9	5.6	5.2	5.7	3.8
9	1536	MS53-89	N	23	24	24	24	8
			Mean	100.8	41.9	18.2	15.0	12.6
			SD	7.8	29.9	14.8	6.4	5.4
10	1463	DP56-89	N	24	24	24	24	8
			Mean	97.9	29.8	8.8	7.4	6.1
			SD	13.7	18.8	5.3	4.2	6.7
11	1692	BH64-90	N	24	24	24	24	8
			Mean	99.4	79.5	70.4	44.8	37.3
			SD	12.6	14.5	27.6	25.6	23.3
12	1691	BH65-90	N	23	23	22	23	8
			Mean	101.7	63.3	38.6	24.7	31.1
			SD	15.7	21.2	24.8	18.5	20.0
13	1689	BH66-90	N	22	24	23	24	8
			Mean	104.4	82.9	59.4	45.0	55.5
			SD	14.2	23.0	27.0	30.3	32.2
14	1690	BH67-90	N	22	24	24	24	8
			Mean	98.0	61.6	40.4	26.8	26.8
			SD	15.3	33.8	26.9	22.3	31.6

* Standard deviation

TASK 89-03, PHASE 1, IN VITRO, TCD
RANKING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY RELATIVE RED BLOOD CELL ACHE
ACTIVITY (%) AT 30 MIN

Order of Means	ICD No.	MREF No.		30 min	Grouping (Means with the same letter are equivalent)
1	1511	MAS4-89	N Mean SD*	24 94.8 16.7	A
2	1469	DP45-89	N Mean SD	24 94.0 14.3	A
3	1465	DP41-89	N Mean SD	24 92.0 17.8	A
4	1466	DP42-89	N Mean SD	24 91.7 18.7	A
5	1689	3M66-90	N Mean SD	24 82.9 23.0	A B
6	1692	3M64-90	N Mean SD	24 79.5 14.5	A B
7	1467	DP43-89	N Mean SD	23 71.2 30.5	A B
8	-	PEG 543	N Mean SD	174 67.6 29.3	B
9	1691	3M65-90	N Mean SD	23 63.3 21.2	B C
10	1690	3M67-90	N Mean SD	24 61.6 33.8	B C
11	1536	MS55-89	N Mean SD	24 41.9 29.9	C D
12	1621	BC61-89	N Mean SD	24 41.4 36.0	C D
13	1463	DP56-89	N Mean SD	24 29.8 18.8	D E
14	1509	BM53-89	N Mean SD	24 20.0 20.3	D E
15	1623	MS163-89	N Mean SD	24 5.2 5.6	E

* Standard deviation.

TASK 89-03, PHASE 1, IN VIVO TGD
RANKING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY RELATIVE RED BLOOD ACHE ACTITITY (%)
AT 60 MIN

Order of Means	ICD No.	MREF No.		60 min	Grouping (Means with the same letter are equivalent)
1	1469	DP45-89	N	24	
			Mean	95.8	A
			SD*	20.8	
2	1465	DP41-89	N	24	
			Mean	87.1	A B
			SD	21.5	
3	1511	MA54-89	N	24	
			Mean	86.5	A B
			SD	14.3	
4	1466	DP42-89	N	24	
			Mean	83.5	A B C
			SD	19.5	
5	1692	3M64-90	N	24	
			Mean	70.6	B C D
			SD	27.6	
6	1689	3M66-90	N	23	
			Mean	59.4	C D E
			SD	27.0	
7	1467	DP43-89	N	24	
			Mean	55.7	D E
			SD	38.3	
8	-	PEG 340	N	176	
			Mean	45.8	E
			SD	28.8	
9	1690	3M67-90	N	24	
			Mean	40.4	E F
			SD	26.9	
10	1691	3M65-90	N	22	
			Mean	38.4	E F
			SD	26.8	
11	1621	BC61-89	N	24	
			Mean	22.3	F G
			SD	21.1	
12	1536	MS55-89	N	24	
			Mean	18.2	F G
			SD	14.8	
13	1463	DP56-89	N	24	
			Mean	8.8	G
			SD	5.3	
14	1509	BM53-89	N	24	
			Mean	8.1	G
			SD	6.8	
15	1623	MS163-89	N	24	
			Mean	3.3	G
			SD	5.2	

* Standard deviation.

TASK 89-03, PHASE 1, IN VIVO, TGD
RANKING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY RELATIVE RED BLOOD CELL ACHE ACTIVITY (%)
AT 120 MIN

Order of Means	ICD No.	MREF No.		120 min	Grouping (Means with the same letter are equivalent)
1	1469	DP45-89	N	24	
			Mean	84.2	A
			SD*	15.1	
2	1511	MAS4-89	N	24	
			Mean	78.9	A
			SD	20.5	
3	1466	DP42-89	N	21	
			Mean	73.6	A
			SD	27.3	
4	1465	DP41-89	N	24	
			Mean	70.2	A
			SD	28.6	
5	1692	3M64-90	N	24	
			Mean	46.8	B
			SD	25.6	
6	1689	3M66-90	N	24	
			Mean	45.0	B
			SD	30.3	
7	1467	DP43-89	N	23	
			Mean	44.0	B
			SD	34.5	
8		PEG 540	N	24	
			Mean	32.6	C
			SD	24.2	
9	1690	3M67-90	N	24	
			Mean	26.8	B C
			SD	22.3	
10	1691	3M65-90	N	23	
			Mean	24.7	B C D
			SD	18.5	
11	1536	MS55-89	N	24	
			Mean	15.0	C D
			SD	6.4	
12	1621	BC61-89	N	24	
			Mean	9.0	C D
			SD	9.1	
13	1509	BM53-89	N	24	
			Mean	6.8	C D
			SD	6.1	
14	1463	DP56-89	N	24	
			Mean	7.4	C D
			SD	4.2	
15	1623	MS163-89	N	24	
			Mean	3.5	D
			SD	5.7	

* Standard Deviation.

MREF TASK 89-03, PHASE 1 IN VIVO, TGD
STATISTICS FOR MEAN RELATIVE ACTIVITY
ACROSS 30-, 60-, AND 120- MIN SAMPLES

Order of Means	ICD No.	MREF No.			Grouping (Means with the same letter are equivalent)
1	1469	DP45-89	N	24	
			Mean	0.913	A
			SD*	0.146	
2	1511	MA54-89	N	24	
			Mean	0.867	A B
			SD	0.144	
3	1465	DP41-89	N	24	
			Mean	0.831	A B C
			SD	0.200	
4	1466	DP42-89	N	21	
			Mean	0.824	A B C
			SD	0.205	
5	1692	3M64-90	N	24	
			Mean	0.656	B C D
			SD	0.209	
6	1689	3M66-90	N	23	
			Mean	0.615	C D E
			SD	0.241	
7	1467	DP43-89	N	22	
			Mean	0.564	D E
			SD	0.355	
8		PEG 540	N	173	
			Mean	0.482	E
			SD	0.254	
9	1690	3M67-90	N	24	
			Mean	0.430	E F
			SD	0.264	
10	1691	3M63-90	N	22	
			Mean	0.414	E F
			SD	0.196	
11	1536	MB55-89	N	24	
			Mean	0.250	F G
			SD	0.159	
12	1621	BC61-89	N	24	
			Mean	0.242	F G
			SD	0.205	
13	1463	DP56-89	N	24	
			Mean	0.153	G
			SD	0.081	
14	1509	BN53-89	N	24	
			Mean	0.116	G
			SD	0.103	
15	1623	MS163-89	N	24	
			Mean	0.040	G
			SD	0.051	

* Standard deviation.

MREF TASK 89-03, PHASE 1,
EVALUATION OF CANDIDATE TOPICAL PROTECTANTS
FOR POSSIBLE ANTI-AChE EFFECTS
INDEXED BY RED BLOOD CELL AChE ACTIVITY (U/mL)
AT 65 AND 5 MIN BEFORE DOSING

Testing Priority	ICD No.	MREF No.		Sample Time Relative To Dosing TOD -65 min	-5 min	Paired Differences**
0		PEG 540	N	175	175	
			Mean	1.96	1.96	0.00
			SD*	0.33	0.39	
1	1466	DP42-89	N	24	24	
			Mean	1.89	1.80	0.09
			SD	0.32	0.45	
2	1467	DP43-89	N	24	23	
			Mean	1.88	1.90	0.01
			SD	0.23	0.38	
3	1465	DP41-89	N	24	24	
			Mean	1.97	1.87	0.10
			SD	0.36	0.30	
4	1469	DP45-89	N	24	24	
			Mean	1.81	1.76	0.05
			SD	0.27	0.28	
5	1511	MA56-89	N	24	24	
			Mean	1.99	2.06	-0.07
			SD	0.29	0.40	
6	1509	BM53-89	N	24	24	
			Mean	1.96	1.96	0.00
			SD	0.34	0.40	
7	1621	BC61-89	N	24	24	
			Mean	1.96	1.95	0.00
			SD	0.42	0.45	
8	1623	MS163-89	N	24	24	
			Mean	1.98	1.93	0.06
			SD	0.36	0.39	
9	1536	MS5-89	N	24	24	
			Mean	1.81	1.85	-0.04
			SD	0.28	0.35	
10	1463	DP56-89	N	24	24	
			Mean	1.86	1.80	0.05
			SD	0.26	0.27	
11	1692	SM64-90	N	24	24	
			Mean	2.14	2.13	0.01
			SD	0.29	0.40	
12	1691	SM65-90	N	23	23	
			Mean	2.04	2.05	-0.02
			SD	0.35	0.40	
13	1689	SM66-90	N	24	22	
			Mean	2.09	2.16	-0.08
			SD	0.36	0.43	
14	1690	SM67-90	N	24	22	
			Mean	2.16	2.15	0.07
			SD	0.33	0.31	

*Standard deviation.
**None were significant ($P < 0.05$, two-sided).

MREF TASK 89-03, PHASE 1,
EVALUATION OF CANDIDATE TOPICAL PROTECTANTS
FOR POSSIBLE LONG-TERM DELAY OF TGD PENETRATION
INDEXED BY RED BLOOD CELL ACHE RELATIVE ACTIVITY (%)
AT 120 MIN AND 24 HR AFTER DOSING

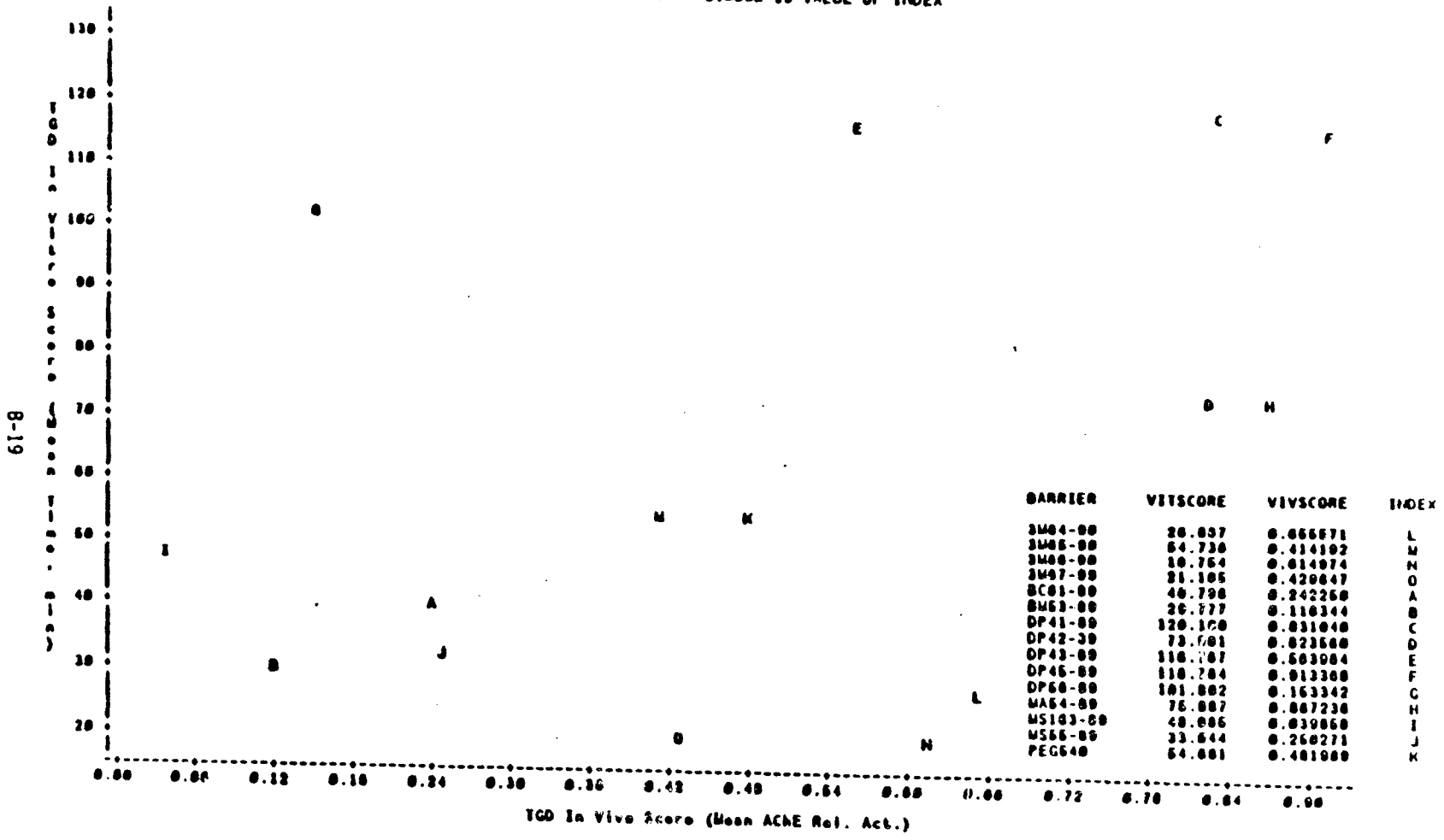
Testing Priority	ICD No.	MREF No.		Sample Time Relative To Dosing TGD 120 min	24 hr	Paired Differences**
0		PEG 540	N	176	56	
			Mean	32.6	30.4	2.0
			SD*	24.2	21.7	
1	1466	DP42-89	N	21	8	
			Mean	73.6	25.1	30.0**
			SD	27.3	22.6	
2	1467	DP43-89	N	23	8	
			Mean	44.0	24.0	9.4**
			SD	34.5	26.6	
3	1465	DP41-89	N	24	8	
			Mean	70.2	68.8	21.8
			SD	28.6	33.6	
4	1469	DP45-89	N	24	8	
			Mean	84.2	85.3	-2.6
			SD	13.1	23.1	
5	1511	HA54-89	N	24	8	
			Mean	78.9	68.9	17.5
			SD	20.5	13.0	
6	1509	BH53-89	N	24	8	
			Mean	6.8	4.6	-0.9
			SD	6.1	6.1	
7	1621	BC61-89	N	24	8	
			Mean	9.3	15.0	-3.7
			SD	9.1	17.9	
8	1623	MB163-89	N	24	8	
			Mean	3.5	1.3	4.7
			SD	5.7	3.8	
9	1536	MB35-89	N	24	8	
			Mean	15.0	12.6	4.1
			SD	6.4	5.4	
10	1463	DP56-89	N	24	8	
			Mean	7.4	6.1	0.8
			SD	4.2	6.7	
11	1692	3M64-90	N	24	8	
			Mean	46.8	37.3	1.6
			SD	25.6	23.3	
12	1691	3M65-90	N	23	8	
			Mean	24.7	31.1	-4.7**
			SD	18.5	20.0	
13	1689	3M66-90	N	24	8	
			Mean	45.0	55.5	-1.0
			SD	30.3	32.2	
14	1690	3M67-90	N	24	8	
			Mean	26.8	26.8	-6.0**
			SD	22.3	31.6	

*Standard deviation.
**Significant ($P < 0.05$, two-sided) paired difference.

TASK 88-83 PHASE 1 TGO IN VITRO RESULTS CORRELATED WITH TGO IN VIVO RESULTS

11:15 MONDAY, JULY 30, 1990

PLOT OF VITSCORE*VIVSCORE SYMBOL IS VALUE OF INDEX



BARRIER	VITSCORE	VIVSCORE	INDEX
BM84-88	28.837	0.666671	L
BM86-88	54.738	0.414102	M
BM88-88	10.764	0.014074	N
BM97-88	31.185	0.420847	O
BC81-88	46.798	0.242268	A
BM83-88	26.777	0.110344	B
DP41-88	120.160	0.831048	C
DP42-30	73.681	0.823688	D
DP43-88	116.787	0.503084	E
DP45-88	118.784	0.813388	F
DP50-88	101.882	0.163342	G
MA54-88	76.887	0.867238	H
MS103-88	48.886	0.039868	I
MS86-88	33.644	0.260271	J
PEG640	64.681	0.481988	K

TASK 89-03, PHASE 1, IN VIVO, HO STATISTICS
FOR CANDIDATE TOPICAL PROTECTANT WEAR TIME
BETWEEN APPLICATION AND DOSING

Candidate Topical Protectant
Wear Time (min)

	N	MEAN	SD*	MINIMUM	MAXIMUM
3M64-90	32	62.3	3.9	60	70
3M65-90	24	62.4	3.6	60	69
3M66-90	24	62.4	3.6	60	69
3M67-90	24	63.0	4.3	60	70
BC61-89	24	86.0	21.0	60	119
BM53-89	23	77.0	12.0	58	99
DP41-89	21	79.4	8.7	54	92
DP42-89	24	78.9	7.8	63	94
DP43-89	24	74.3	6.4	62	86
DP43-89	23	88.2	12.6	65	110
DP56-89	24	82.6	19.4	60	113
MA54-89	23	88.2	12.6	65	110
MS163-89	24	86.0	21.0	60	119
MS55-89	24	82.6	19.4	60	113
PEG 540	124	73.4	10.3	54	99

* Standard deviation.

MREF TASK 89-03, PHASE 1
IN VIVO ASSESSMENT OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS

Testing Priority	ICD No.	MREF No.		Time After Dosing to Decontamination 1 hr	2 hr	4 hr
0	-	PEG 540	N	116	116	116
			Mean	176.4	225.5	285.2
			SD*	90.8	95.5	122.5
1	1466	DP42-89	N	24	24	24
			Mean	48.4	77.2	123.4
			SD	19.0	30.7	41.3
2	1467	DP43-89	N	24	24	24
			Mean	65.5	122.8	155.5
			SD	60.4	57.8	66.8
3	1465	DP41-89	N	21	21	21
			Mean	26.0	47.2	83.0
			SD	18.8	35.0	55.4
4	1469	DP45-89	N	23	23	23
			Mean	19.7	44.0	68.9
			SD	12.2	26.0	39.3
5	1511	MA54-89	N	23	23	23
			Mean	25.9	44.1	66.0
			SD	17.9	24.9	34.3
6	1509	BM53-89	N	23	23	23
			Mean	334.0	386.1	482.4
			SD	195.7	184.6	203.7
7	1621	BC61-89	N	24	24	24
			Mean	110.0	175.6	216.3
			SD	57.7	79.2	89.0
8	1623	MS163-89	N	24	24	24
			Mean	454.7	476.9	545.1
			SD	152.7	166.4	157.0
9	1536	MS55-89	N	24	24	24
			Mean	57.5	79.6	141.3
			SD	98.7	111.5	182.4
10	1463	DP56-89	N	24	24	24
			Mean	62.3	107.4	157.5
			SD	32.6	66.5	92.7
11	1692	3M64-90	N	24	24	24
			Mean	52.9	102.7	163.7
			SD	32.1	71.9	91.7
12	1691	3M65-90	N	24	24	24
			Mean	39.7	64.8	111.0
			SD	29.8	37.9	63.0
13	1689	3M66-90	N	24	24	24
			Mean	30.3	43.6	67.3
			SD	18.1	25.1	46.1
14	1690	3M67-90	N	24	24	24
			Mean	75.2	116.8	174.0
			SD	38.2	57.9	80.6

* Standard deviation.

MREF TASK 89-03, PHASE 1
IN VIVO ASSESSMENT OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY NO LESION AREAS RELATIVE TO UNPROTECTED SITE (%)
RESULTING FROM THREE EXPOSURE PERIODS

Testing Priority	ICD No.	MREF No.		Time After Dosing to Decontamination 1 hr	2 hr	4 hr	Score**
0	-	PEG 540	N	116	116	116	116
			Mean	38.6	50.3	63.7	0.509
			SD*	15.2	15.8	21.7	0.158
1	1466	DP42-89	N	24	24	24	24
			Mean	9.9	15.3	25.8	0.170
			SD	5.0	6.2	14.1	0.080
2	1467	DP43-89	N	24	24	24	24
			Mean	14.3	25.2	31.8	0.238
			SD	17.3	10.2	9.6	0.090
3	1465	DP41-89	N	21	21	21	21
			Mean	5.3	9.4	18.0	0.109
			SD	3.7	5.7	14.5	0.066
4	1469	DP45-89	N	23	23	23	23
			Mean	5.1	13.2	18.9	0.124
			SD	2.6	11.0	10.2	0.069
5	1511	MAS4-89	N	23	23	23	23
			Mean	7.2	12.9	18.9	0.130
			SD	5.1	10.0	14.3	0.089
6	1509	BM53-89	N	23	23	23	23
			Mean	79.3	93.3	116.6	0.964
			SD	37.9	32.7	34.1	0.299
7	1621	BC61-89	N	24	24	24	24
			Mean	26.0	41.3	51.6	0.396
			SD	14.4	25.7	31.3	0.215
8	1427	MS163-89	N	24	24	24	24
			Mean	109.3	113.7	132.9	1.186
			SD	54.7	55.8	42.7	0.566
9	1536	MS55-89	N	24	24	24	24
			Mean	11.0	16.9	28.4	0.188
			SD	12.9	18.5	28.8	0.108
10	1463	DP56-89	N	24	24	24	24
			Mean	14.9	25.3	35.7	0.253
			SD	7.7	13.2	15.5	0.108
11	1692	3M64-90	N	24	24	24	24
			Mean	18.7	29.3	43.1	0.304
			SD	27.4	22.6	17.2	0.175
12	1691	3M65-90	N	24	24	24	24
			Mean	9.3	15.0	26.6	0.170
			SD	5.2	5.6	13.2	0.069
13	1689	3M66-90	N	24	24	24	24
			Mean	7.2	10.6	15.6	0.111
			SD	3.2	5.0	7.5	0.047
14	1690	3M67-90	N	24	24	24	24
			Mean	16.9	26.3	37.5	0.269
			SD	9.1	10.7	9.6	0.080

* Standard deviation.
** Mean of 1-, 2-, and 4- hr relative areas, expressed as a fraction.

MREF TASK 89-03, PHASE 1
ORDERING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%)
TIME TO DECONTAMINATION: 1 hr

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)
1	1469	DP45-89	N	23	
			Mean	5.1	A
			SD*	2.6	
2	1465	DP61-89	N	21	
			Mean	5.3	A B
			SD	3.7	
3	1689	3M66-90	N	24	
			Mean	7.2	A B
			SD	3.2	
4	1511	MA54-89	N	23	
			Mean	7.2	A B
			SD	5.1	
5	1691	3M65-90	N	24	
			Mean	9.3	A B
			SD	5.2	
6	1466	DP42-89	N	24	
			Mean	9.9	A B
			SD	5.0	
7	1536	MB55-89	N	24	
			Mean	11.0	A B
			SD	12.9	
8	1467	DP43-89	N	24	
			Mean	14.3	A B
			SD	17.3	
9	1463	DP56-89	N	24	
			Mean	14.9	A B
			SD	7.7	
10	1690	3M67-90	N	24	
			Mean	16.9	A B
			SD	9.1	
11	1692	3M64-90	N	24	
			Mean	18.7	A B
			SD	27.4	
12	1621	BC61-89	N	24	
			Mean	26.0	B C
			SD	14.4	
13	-	PEG 540	N	116	
			Mean	38.6	C
			SD	15.2	
14	1509	BM53-89	N	23	
			Mean	79.3	D
			SD	37.9	
15	1623	MA163-89	N	24	
			Mean	109.3	E
			SD	54.7	

* Standard deviation.

MREF TASK 89-03, PHASE 1
ORDERING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%)
TIME TO DECONTAMINATION: 2 hr

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)
1	1465	DP41-89	N	21	
			Mean	9.4	A
			SD*	5.7	
2	1689	3M66-90	N	24	
			Mean	10.4	A
			SD	5.0	
3	1511	MA54-89	N	23	
			Mean	12.9	A
			SD	10.0	
4	1469	DP45-89	N	23	
			Mean	13.2	A
			SD	11.0	
5	1691	3M65-90	N	24	
			Mean	15.0	A
			SD	5.6	
6	1466	DP42-89	N	24	
			Mean	15.3	A
			SD	6.2	
7	1536	MS55-89	N	24	
			Mean	16.9	A
			SD	8.5	
8	1467	DP43-89	N	24	
			Mean	25.2	A B
			SD	10.2	
9	1463	DP56-89	N	24	
			Mean	25.3	A B
			SD	13.2	
10	1690	3M67-90	N	24	
			Mean	26.3	A B
			SD	10.7	
11	1692	3M64-90	N	24	
			Mean	29.3	A B
			SD	22.6	
12	1621	BC61-89	N	24	
			Mean	41.3	B C
			SD	23.7	
13	-	PEG 540	N	116	
			Mean	50.3	C
			SD	15.8	
14	1509	BM53-89	N	23	
			Mean	93.3	D
			SD	32.7	
15	1623	MS163-89	N	24	
			Mean	113.7	D
			SD	55.8	

* Standard deviation.

MREF TASK 89-03, PHASE 1
ORDERING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY NO LESION AREAS RELATIVE TO UNPROTECTED SITE (%)
TIME TO DECONTAMINATION: 4 hr

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)
1	1689	3M66-90	N	24	
			Mean	15.6	A
			SD*	7.5	
2	1465	DP41-89	N	21	
			Mean	18.0	A B
			SD	14.5	
3	1511	MA54-89	N	23	
			Mean	18.9	A B
			SD	14.3	
4	1469	DP45-89	N	23	
			Mean	18.9	A B
			SD	10.2	
5	1466	DP42-89	N	24	
			Mean	25.8	A B
			SD	16.1	
6	1691	3M65-90	N	24	
			Mean	26.6	A B
			SD	13.2	
7	1536	MS55-89	N	24	
			Mean	28.6	A B C
			SD	28.8	
8	1467	DP43-89	N	24	
			Mean	31.8	A B C
			SD	9.6	
9	1463	DP56-89	N	24	
			Mean	35.7	A B C
			SD	15.5	
10	1690	3M67-90	N	24	
			Mean	37.5	A B C
			SD	9.6	
11	1692	3M64-90	N	24	
			Mean	43.1	B C
			SD	17.2	
12	1621	BC61-89	N	24	
			Mean	51.6	C D
			SD	31.3	
13	-	PEG 540	N	116	
			Mean	63.7	D
			SD	21.7	
14	1509	BM53-89	N	23	
			Mean	116.6	E
			SD	34.1	
15	1623	MS163-89	N	24	
			Mean	132.9	E
			SD	62.7	

* Standard deviation.

MREF TASK 89-03, PHASE 1
ORDERING OF CANDIDATE TOPICAL PROTECTANTS
INDEXED BY MEAN OF NO LESION AREAS AT 1, 2, AND 4 HR
RELATIVE TO UNPROTECTED SITE, EXPRESSED AS A FRACTION

Order of Means	ICD No.	MREF No.		Relative Area	Grouping (Means with the same letter are equivalent)
1	1465	DP41-89	N	21	
			Mean	0.109	A
			SD*	0.066	
2	1689	3M66-90	N	24	
			Mean	0.111	A
			SD	0.047	
3	1469	DP45-89	N	23	
			Mean	0.124	A
			SD	0.069	
4	1511	MA54-89	N	23	
			Mean	0.130	A
			SD	0.089	
5	1691	3M65-90	N	24	
			Mean	0.170	A
			SD	0.069	
6	1466	DP42-89	N	24	
			Mean	0.170	A
			SD	0.080	
7	1536	MS55-89	N	24	
			Mean	0.188	A
			SD	0.188	
8	1467	DP43-89	N	24	
			Mean	0.238	A B
			SD	0.090	
9	1463	DP56-89	N	24	
			Mean	0.253	A B
			SD	0.108	
10	1690	3M67-90	N	24	
			Mean	0.269	A B
			SD	0.060	
11	1692	3M64-90	N	24	
			Mean	0.304	A B
			SD	0.175	
12	1621	BC61-89	N	24	
			Mean	0.396	B C
			SD	0.215	
13	-	PEG 540	N	116	
			Mean	0.509	C
			SD	0.158	
14	1509	BM53-89	N	23	
			Mean	0.966	D
			SD	0.299	
15	1623	MS163-89	N	24	
			Mean	1.186	E
			SD	0.566	

* Standard deviation.

APPENDIX C

Letter Report on Phase 2, Functional Testing, Dated 9 January, 1991

Review and Approval

No. [illegible]

	Name	Initials	Date
Originator	DW Hobson		
Concurrence			
Approved	CS Dill See Draft		

Internal Distribution
DW Hobson
RMO
CS Dill/File

January 9, 1991

LTC Don W. Korte, Jr., MS, COR
Battelle Columbus Operations
505 King Avenue - JM-3
Columbus, Ohio 43201-2693

Dear LTC Korte:

Contract DAMD-17-89-C-9050
Task 89-03 Letter Report

Attached are statistically analyzed summaries of the results from Task 89-03, phase two studies in which rabbits were coated with a 0.1 mm thick layer of a candidate topical skin protectant (TSP) before topical application of either 1 µL of HD or 3.35 mg/kg of TGD. Phase two studies were performed as in phase one, using either TGD or HD challenges and included either a water stressing step to assess the TSP efficacy after washing the application site with a quantity of water equivalent to 500 times the volume of TSP applied, or a time stressing step (to assess the TSP efficacy after a test-specified wear period of 4 hr).

A mixture of polyethylene glycols with an average molecular weight of 540 daltons (PEG 540) was the control TSP in each test. Student's unpaired t test (alpha = 0.05, two-sided) was performed to determine whether the differences between unstressed versus water-stressed and unstressed versus time-stressed TSP performance were significant.

For all HD tests, the endpoint used for statistical comparisons is the lesion area ratio expressed as a percentage of a control lesion area (no protection) produced on each rabbit. From the water-stressed studies, results are included for both a 1- and 2-hr period between dosing HD and decontaminating the dose site with a 5 percent sodium hypochlorite (NaOCl) solution.

The endpoint used to statistically assess TSP efficacy against TGD is the inhibition of erythrocyte acetylcholinesterase (AChE) activity expressed as a percentage of pre-TSP application baseline activity. Blood samples were collected at 65 (baseline) and 5 (TSP wear time = 60 min) min before application and at 30, 60, 120, and 1440 min after TGD application.

Initially, five TSPs were recommended by U.S. Army Medical Research Institute for Chemical Defense (USAMRICD) investigators for phase two testing based on

their phase one performance. However, in response to a mid-phase change received from USAMRICD, 6 September 1990, only three of the original five TSPs were to be tested in phase two, i.e., ICD Nos. (MREF Nos.) 1536 (MS55-89), 1511 (MA54-89), and 1465 (DP41-89). At the time of the request, nearly all water-stressing tests had been completed for the original five compounds. Thus, in response to the request, no time-stress studies were performed with ICD Nos. (MREF Nos.) 1466 (DP42-89) and 1469 (DP45-89).

HD Results

Absolute lesion area ratios for all phase two HD studies are summarized and presented as univariate statistical parameters in Table 1. Univariate statistics for HD lesion area ratios (LARs) expressed as a percentage of the control dose site on the same rabbit are shown in Table 2. Results in the "None" and "Time" columns for stress are from rabbits that received 1 µL of HD per dose site, at either 1 hr or 4 hr after the TSP was spread, respectively. Results in the "Water' column are from rabbits that had TSP applied and then the treatment sites were rinsed with water 1 hr prior to HD application. An increase in the mean LAR estimated for a stressed TSP relative to its unstressed mean LAR indicates decreased TSP efficacy. Such decreases were considered significant ($P < 0.05$) depending on the outcomes of unpaired t tests.

Of the three time-stressed TSPs, only ICD No. 1536 efficacy was significantly ($P < 0.05$) decreased by the 4-hr time stress. The mean LAR for ICD No. 1536 increased, no stress relative to time-stressed, from 11 to 58 and 17 to 71 percent for 1- and 2-hr HD exposure periods, respectively. ICD No. 1536 efficacy was also adversely affected by water stressing, with mean LARs increasing, no stress relative to water-stressed, from 11 to 36 and 17 to 64 percent for 1- and 2-hr HD exposure periods, respectively.

ICD No. 1466 efficacy was significantly ($P < 0.05$) enhanced by water-stressing at both times to decontamination, with mean LARs decreasing, no stress relative to water-stress, from 9.9 to 4.9 and 15.3 to 7.7 percent, respectively. ICD No. 1511 became slightly more efficacious following water stressing at the 2-hr HD exposure period (13 to 9 percent change), however the significance here was nearly equivocal ($P = 0.0462$). There were no other significant effects due to either time- or water-stressing observed for any of the other TSPs tested against HD.

TGD Results

A univariate statistical summary for absolute rabbit erythrocyte AChE activity results from all TGD tests is shown in Table 3. Univariate statistics for erythrocyte AChE activity, calculated as a percentage of the baseline level in the same rabbit are shown in Table 4. An increase in the mean activity level

relative to unstressed controls indicates increased TSP efficacy, depending on the statistical significance of the difference.

Time-stressing significantly ($P < 0.05$) decreased the efficacy of ICD No. 1465 against TGD at the 120-min sample time; the mean relative AChE activity levels for unstressed versus time-stressed groups were 70 and 49 percent, respectively. However, time-stressing ICD No. 1536 apparently significantly ($P < 0.05$) enhanced early protection against TGD; at the 30- and 60-min sample times, the mean relative activity levels (unstressed versus time-stressed) were 42 versus 64 percent and 18 versus 32 percent, respectively. By 120 min the mean relative activity levels were the same.

Water-stressing ICD No. 1465 also significantly decreased its efficacy against TGD, but only at the 60-min sample time (87 versus 74 percent). The benefit of water-stressing ICD No. 1466 was not apparent until 24 hr after dosing, when the mean activity level was maintained at 65 percent (versus 25 percent for the unstressed controls). Pursuant to the mid-task directive limiting the scope of this task, water-stress testing ICD No. 1469 was halted after eight rabbits had been used. There was no apparent explanation for why there was a significant beneficial effect at 30 min from water stressing ICD No. 1469, since the mean relative activity level was 20 percent above the baseline controls. There were no other significant effects due to either time- or water-stressing any of the TSPs tested against TGD in phase two.

If I can be of further assistance in the interpretation or clarification of these findings, please contact me at (614) 424-5259.

Sincerely,

David W. Hobson

David W. Hobson, Ph.D., D.A.B.T.
Associate Manager
Medical Research and Evaluation Facility

DWH/cah

Attachments

cc: COL Michael A. Dunn, Commander, USAMRICD
LTC George C. Southworth, Deputy Commander, USAMRICD
COL Douglas Reichard, MS, RAD V, USAMRDC
LTC James R. Stewart, VC, USAMRICD
Ms. Ellen Mackenzie, Chief, PCMB, USAMRICD

TABLE 1. PHASES 1 AND 2, IN VIVO, HD STATISTICS FOR ABSOLUTE AREA (sq. mm) BY TIME (min) AFTER DOSING HD

TSP ICD No.	TSP MREF No.	Time (hr) to Decontamination		Type of Stress: None	Type of Stress: Time	Type of Stress: Water
1465	DP41-89	1	N	21	21	22
			MEAN	26.0	27.7	28.4
			STD	18.8	20.9	16.9
		2	N	21	21	22
			MEAN	47.2	46.4	38.5
			STD	35.0	24.6	22.2
1466	DP42-89	1	N	24	-	24
			MEAN	48.4	-	19.5
			STD	19.0	-	6.3
		2	N	24	-	24
			MEAN	77.2	-	29.9
			STD	30.7	-	16.3
1469	DP45-89	1	N	23	-	24
			MEAN	19.7	-	19.1
			STD	12.2	-	8.6
		2	N	23	-	24
			MEAN	44.0	-	33.6
			STD	24.0	-	31.2
1511	MA54-89	1	N	23	22	22
			MEAN	25.9	41.2	27.0
			STD	17.9	29.4	17.8
		2	N	23	22	22
			MEAN	44.1	57.3	36.6
			STD	24.9	21.6	22.9
1536	MS55-89	1	N	24	22	22
			MEAN	57.5	256.4	153.8
			STD	98.7	155.2	130.3
		2	N	24	22	22
			MEAN	79.6	311.1	253.6
			STD	111.5	146.8	151.5
-	PEG 540	1	N	167	-	-
			MEAN	174.5	-	-
			STD	84.1	-	-
		2	N	167	-	-
			MEAN	227.1	-	-
			STD	100.5	-	-

TABLE 2. TASK 89-03 PHASES 1 AND 2, IN VIVO,
STATISTICS FOR HD LESION AREA RATIOS (%)
BY TIME TO DECONTAMINATION

TSP		Time (hr) to Decontamination		Type of Stress		
ICO No.	MREF No.			None	Time	Water
1465	DP41-89	1	N	21	21	22
			MEAN	5.3	6.8	7.1
			STD	3.7	4.8	4.4
		2	N	21	21	22
			MEAN	9.4	13.5	10.3
			STD	5.7	12.5	8.9
1466	DP42-89	1	N	24	-	24
			MEAN	9.9	-	4.9*
			STD	5.0	-	1.8
		2	N	24	-	24
			MEAN	15.3	-	7.7*
			STD	6.2	-	4.6
1469	DP45-89	1	N	23	-	24
			MEAN	5.1	-	5.0
			STD	2.6	-	2.8
		2	N	23	-	24
			MEAN	13.2	-	10.0
			STD	11.0	-	15.3
1511	MA54-89	1	N	23	22	22
			MEAN	7.2	8.9	6.9
			STD	5.1	4.0	5.5
		2	N	23	22	22
			MEAN	12.9	13.7	8.7*
			STD	10.0	6.0	4.3
1536	MS55-89	1	N	24	22	22
			MEAN	11.0	57.6*	35.6*
			STD	12.9	23.3	24.7
		2	N	24	22	22
			MEAN	16.9	71.3*	64.2*
			STD	18.5	28.5	48.6
-	PEG 540	1	N	167	-	-
			MEAN	40.2	-	-
			STD	16.0	-	-
		2	N	167	-	-
			MEAN	52.8	-	-
			STD	21.3	-	-

* Significant ($P < 0.05$, two-sided) effect due to stress at the respective time to decontamination (HD exposure period).

TABLE 3. PHASES 1 AND 2, IN VIVO, STATISTICS FOR ABSOLUTE AChE ACTIVITY (U/mL) BY TIME (min) AFTER DOSING TGD

TSP ICD No.	TSP MREF No.	Sample Time (min) After Dosing		Type of Stress None	Type of Stress Time	Type of Stress Water
1465	DP41-89	-65	N	24	24	23
			MEAN	1.97	2.24	2.04
			STD	0.36	0.32	0.39
		-5	N	24	23	23
			MEAN	1.87	2.22	2.01
			STD	0.30	0.30	0.38
		30	N	24	24	23
			MEAN	1.79	2.01	1.84
			STD	0.38	0.31	0.51
		60	N	24	24	23
			MEAN	1.68	1.70	1.49
			STD	0.36	0.54	0.45
		120	N	24	24	23
			MEAN	1.35	1.08	1.23
			STD	0.51	0.46	0.53
		1,440	N	8	8	7
			MEAN	1.14	1.39	1.36
			STD	0.58	0.31	0.61
1466	DP42-89	-65	N	24	.	24
			MEAN	1.89	.	2.15
			STD	0.32	.	0.39
		-5	N	24	.	24
			MEAN	1.80	.	2.08
			STD	0.45	.	0.51
		30	N	24	.	24
			MEAN	1.70	.	1.99
			STD	0.36	.	0.38
		60	N	24	.	24
			MEAN	1.55	.	1.70
			STD	0.31	.	0.62
		120	N	21	.	24
			MEAN	1.33	.	1.61
			STD	0.45	.	0.64
		1,440	N	8	.	8
			MEAN	0.48	.	1.56
			STD	0.46	.	0.38

TABLE 3.
(Continued)

TSP ICD No.	TSP MREF No.	Sample Time (min) After Dosing		Type of Stress None	Type of Stress Time	Type of Stress Water
1469	DP45-89	-65	N	24	.	8
			MEAN	1.81	.	2.22
			STD	0.27	.	0.42
		-5	N	24	.	8
			MEAN	1.76	.	2.59
			STD	0.28	.	0.42
		30	N	24	.	8
			MEAN	1.68	.	2.64
			STD	0.22	.	0.46
		60	N	24	.	8
			MEAN	1.71	.	2.42
			STD	0.32	.	0.31
		120	N	24	.	8
			MEAN	1.52	.	2.04
			STD	0.32	.	0.44
		1,440	N	8	.	0
			MEAN	1.46	.	.
			STD	0.47	.	.
1511	MA54-89	-65	N	24	24	24
			MEAN	1.99	2.16	2.23
			STD	0.29	0.41	0.35
		-5	N	24	24	23
			MEAN	2.05	2.19	2.25
			STD	0.40	0.43	0.45
		30	N	24	24	24
			MEAN	1.89	2.11	2.05
			STD	0.42	0.39	0.37
		60	N	24	24	24
			MEAN	1.73	2.02	2.10
			STD	0.41	0.46	0.44
		120	N	24	24	24
			MEAN	1.57	1.57	1.83
			STD	0.42	0.44	0.50
		1,440	N	8	8	8
			MEAN	1.38	1.29	1.76
			STD	0.44	0.38	0.20

TABLE 3.
(Continued)

TSP ICD No.	TSP MREF No.	Sample Time (min) After Dosing		Type of Stress: None	Time	Water
1536	MS55-89	-65	N	24	23	24
			MEAN	1.82	2.19	2.18
			STD	0.28	0.29	0.37
		-5	N	23	23	24
			MEAN	1.80	2.25	2.21
			STD	0.29	0.33	0.36
		30	N	24	23	24
			MEAN	0.77	1.41	0.71
			STD	0.59	0.76	0.49
		60	N	24	23	24
			MEAN	0.34	0.72	0.37
			STD	0.32	0.59	0.27
		120	N	24	23	24
			MEAN	0.27	0.36	0.25
			STD	0.13	0.44	0.24
		1,440	N	8	8	7
			MEAN	0.25	0.38	0.28
			STD	0.10	0.18	0.28
	PEG 540	-65	N	317	.	.
			MEAN	2.05	.	.
			STD	0.37	.	.
		-5	N	313	.	.
			MEAN	2.08	.	.
			STD	0.43	.	.
		30	N	316	.	.
			MEAN	1.39	.	.
			STD	0.64	.	.
		60	N	315	.	.
			MEAN	0.96	.	.
			STD	0.59	.	.
		120	N	315	.	.
			MEAN	0.64	.	.
			STD	0.46	.	.
		1,440	N	99	.	.
			MEAN	0.64	.	.
			STD	0.42	.	.

TABLE 4: PHASES 1 AND 2, IN VIVO, STATISTICS FOR AChE ACTIVITY RELATIVE TO BASELINE (%) BY TIME (min) AFTER DOSING TGD

TSP		Sample Time (min) After Dosing		Type of Stress		
ICD No.	MREF No.			None	Time	Water
1465	DP41-89	30	N	24	24	23
			MEAN	92.0	90.7	90.8
			STD	17.8	15.8	18.6
		60	N	24	24	23
			MEAN	87.1	76.9	74.1*
			STD	21.5	24.9	20.9
		120	N	24	24	23
			MEAN	70.2	49.1*	60.7
			STD	28.6	22.1	26.5
		1,440	N	8	8	7
			MEAN	68.8	58.5	55.0
			STD	33.6	14.7	21.6
1466	DP42-89	30	N	24	-	24
			MEAN	91.7	-	92.0
			STD	18.7	-	18.5
		60	N	24	-	24
			MEAN	83.5	-	77.7
			STD	19.5	-	21.7
		120	N	21	-	24
			MEAN	73.4	-	73.4
			STD	27.3	-	23.4
		1,440	N	8	-	8
			MEAN	25.1	-	65.1*
			STD	22.6	-	11.7
1469	DP45-89	30	N	24	-	8
			MEAN	94.0	-	119.6*
			STD	14.3	-	11.5
		60	N	24	-	8
			MEAN	95.8	-	110.8
			STD	20.8	-	14.3
		120	N	24	-	8
			MEAN	84.2	-	91.9
			STD	15.1	-	9.2
		1,440	N	8	-	0
			MEAN	85.3	-	-
			STD	23.1	-	-

TABLE 4.
(Continued)

TSP ICD No.	TSP MRER No.	Sample time (min) After Dosing		Type of Stress None	Time	Water
1511	MA54-89	30	N	24	24	24
			MEAN	94.8	98.3	91.6
			STD	14.7	10.2	9.6
		60	N	24	24	24
			MEAN	86.5	94.2	94.3
			STD	14.3	16.5	15.1
		120	N	24	24	24
			MEAN	78.9	73.3	81.6
			STD	20.5	17.3	17.0
		1,440	N	8	8	8
			MEAN	68.9	56.3	81.2
			STD	13.0	13.5	11.6
1536	MS55-89	30	N	24	23	24
			MEAN	41.9	63.5*	34.0
			STD	29.9	30.9	25.4
		60	N	24	23	24
			MEAN	18.2	32.0*	17.7
			STD	14.8	24.1	13.7
		120	N	24	23	24
			MEAN	15.0	15.7	11.9
			STD	6.4	17.6	11.2
		1,440	N	8	8	7
			MEAN	12.6	18.0	15.6
			STD	5.4	8.8	16.3
-	PEG 540	30	N	316	-	-
			MEAN	68.2	-	-
			STD	29.6	-	-
		60	N	315	-	-
			MEAN	47.3	-	-
			STD	29.0	-	-
		120	N	315	-	-
			MEAN	32.0	-	-
			STD	23.5	-	-
		1,440	N	99	-	-
			MEAN	31.3	-	-
			STD	20.2	-	-

*Significant ($P < 0.05$, two-sided) effect due to stress at the respective sample time.

APPENDIX D

Letter Report on Phase 3, Advanced Efficacy Testing, Dated 24 June 1991

For Review and Approval

No. G1555-[illegible]
Internal Distribution

Name	Initials	Date
Originator DW Hobson	[illegible]	6/24
Concurrence		
Approval GS Dill	[illegible]	6/25

June 24, 1991

DW Hobson
TH Snider
RMO
GS Dill/File

LTC Don W. Korte, Jr., MS, COR
Battelle Columbus Operations
505 King Avenue, JM-3
Columbus, OH 43201-2693

Dear LTC Korte:

Contract DAMD17-89-C-9050
Task 89-03 (Phase Three) Letter Report

The attached document is a statistically analyzed, summary of the results from Medical Research and Evaluation Facility (MREF) Task 89-03 Phase Three studies in which rabbits were treated with a 0.1 mm thick layer of a candidate topical skin protectant (TSP) before challenge with either 1 µL of HL, 1.35 mg/kg of GD or 0.30 mg/kg of VX. The summarized test results from phases one and two have been previously reported. MREF Task 89-03 phase one studies were performed using both in vitro and in vivo procedures. Phase one in vitro tests were performed using GD, TGD, and VX challenges and phase one in vivo tests included challenges to either HD or TGD. Phase two studies were performed in vivo only with HD and TGD challenges and included either a water stressing step in order to assess the TSP efficacy in a high-moisture environment, or a time stressing step in order to assess the TSP efficacy after 4-hr wear period. In all phases, the rabbit has been the animal model used for in vivo testing, and the control TSP has been a mixture of polyethylene glycols having a mean molecular weight of 540 daltons (PEG 540).

The mixture of HD and L used to produce HL was 75 percent HD and 25 percent L by volume. The endpoint reported for HL tests is the lesion area expressed as a percentage of a control lesion area (no protection) for each rabbit.

The endpoint reported for GD and VX is the red blood cell acetylcholinesterase (AChE) activity expressed on an individual animal basis, as a percentage of pre-TSP application baseline value. Blood samples were collected at 65 (baseline) and 5 min before application (-65 and -5 min, respectively) and at 30, 60, 120, and 1,440 min after application of agent. For GD, VX, and HL tested in vivo this document includes the following information:

a. Univariate statistics on the raw endpoint measured at each time period, i.e., red blood cell acetylcholinesterase (AChE) activity (U/mL) for GD and VX, and lesion area (mm^2) for HL. In accordance with MREF Protocol 58, animals that died before a blood sample was collected, were arbitrarily assigned an AChE activity value of zero for post-mortem collection periods. The number of dead animals are indicated in parenthesis in Table 9 for VX challenge (none died from GD challenge).

b. Univariate statistics on the relative endpoint calculated at each time period, i.e., red blood cell AChE activity divided by the baseline value (%) for GD and VX, and lesion area divided by the unprotected control site lesion area (%) for HL,

c. Descriptive statistics on the relative endpoint at each time period, ordered from apparent most to least effective TSP. These tables identify groups of TSPs having statistically indistinguishable means, determined by analysis of variance with the least-squares means method (Statistical Analysis System General Linear Models, or SAS GLM, procedure.) In each case the decision level was set at alpha = 0.05.

d. Descriptive statistics on the mean relative endpoint averaged across time periods by rabbit, expressed as a fraction, and ordered from apparent most to least effective TSP; this table identifies groups of TSPs having statistically indistinguishable means, determined by analysis of variance (alpha = 0.05) with the least-squares means method (SAS GLM procedure).

e. For GD and VX only, descriptive statistics and paired t-tests to determine the effect of each TSP on rabbit AChE absolute activity from just before TSP application (-65 min) to 1 hr later (-5 min).

f. For GD and VX only, descriptive statistics and paired t-tests to determine whether rabbit AChE relative activity levels changed from 120 min to 24 hr after dosing. In these tables, the paired differences are generally not the same as the differences between the 120-min and 24-hr mean levels shown. These differences are due to the fact that on only one of the three replicate days were the rabbits held for 24 hr, so paired differences were determined for only eight rabbits and not for all 24.

Results of In Vivo Tests Involving GD (Tables 1-8)

Performances of the PEG 540 control and three TSPs tested in Phase Three were statistically ($P < 0.05$, two-sided) distinguishable when tested against GD. At each of the three blood sample times immediately after dosing, ICD Nos. 1465 and 1511 were statistically superior to ICD No. 1536 and PEG 540 in sustaining protection against AChE inhibition. The lowest mean relative AChE activity level estimated for ICD No. 1511 was 76.9 percent of the baseline at 120 min after dosing. Mean relative activity levels averaged by rabbit across 30-, 60-, and 120-min blood sample times indicated this order of TSP efficacy against GD: ICD Nos. 1511 = 1465 > PEG 540 = ICD No. 1536.

Paired t tests comparing pre-TSP application AChE levels (-65 min) with those 1 hr later (-5 min) indicated no change in activity for any of the TSPs ($P > 0.05$, two-sided). Paired t tests between relative AChE activity levels at 120 min and 24 hr indicated significant ($P < 0.05$, two-sided) recoveries in activity levels for animals receiving TSPs PEG 540 (7.8 percent) and ICD No. 1511 (13.4 percent). These differences are not equal to the differences between the means of all rabbits because they were paired by each rabbit used in the overnight studies only.

Results of In Vivo Tests Involving VX (Tables 9-16)

The relative performances of the TSPs were markedly different with the VX challenge versus what was discussed above for the GD challenge. Notably, ICD No. 1465 offered the poorest protection at all three critical blood sample times. ICD Nos. 1511 and 1536 were statistically ($P < 0.05$, two-sided) better than PEG 540 and ICD No. 1465 at 30 and 60 min, but only ICD No. 1511 was statistically distinguishable as superior at 120 min after dosing. Mean relative activity levels averaged by rabbit across 30-, 60-, and 120-min blood sample times indicated this order of TSP efficacy against VX: ICD Nos. 1511 > 1536 > PEG 540 = ICD No. 1465.

Paired t tests comparing pre-TSP application AChE levels (-65 min) with those 1 hr later (-5 min) indicated no change in activity for any of the TSPs ($P > 0.05$, two-sided). Paired t tests between relative AChE activity levels at 120 min and 24 hr indicated a significant ($P < 0.05$, two-sided) decrease in activity levels for animals receiving TSP ICD No. 1536 (5.2 percent). This decrease indicated the possibility of continued penetration of VX through ICD No. 1536 when left on the skin for more than 2 hr.

Results of In Vivo Tests Involving HL (Tables 17-22)

Relative performances of three TSPs and PEG 540 against HL challenge were similar to that against GD. Topical application of ICD Nos. 1511 and 1465 resulted in the greatest protection, i.e., low mean relative lesion areas at each of the HL exposure periods, 1, 2, and 4 hr. Mean relative area averaged across exposure periods indicated this order of TSP efficacy: ICD Nos. 1511 = 1465 > PEG 540 = ICD No. 1536.

Discussion

Based on the TSP mean performance indices averaged across exposure periods, ICD 1511 (MREF MA54-89) performed the best against all three agent challenges, with mean relative AChE activity levels (for GD and VX) and mean relative lesions areas (for HL) statistically distinct from those for PEG 540 and ICD No. 1536 (MREF MS55-89). Of the blood samples we collected, the lowest AChE activity levels allowed by ICD No. 1511 were 1.78 U/mL at 120 min after dosing GD (76.9 percent of the pre-TSP application baseline) and 1.33 U/mL at 24 hr after dosing VX (60.5 percent of the pre-TSP application baseline).

ICD No. 1511 allowed lesion areas of only 11.2 percent of those at unprotected control sites.

If further clarification of these findings is desired or if I can be of any further assistance, please contact me at (614) 424-5259.

Sincerely,

David W. Hobson, Ph.D., D.A.B.T.
Research Leader

DWH/cah

Attachment

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Ms. Ellen Mackenzie, Chief, PCMB, USAMRICD

TABLE 1. MREF TASK 89-03, PHASE 3, IN VIVO, GD
STATISTICS FOR ABSOLUTE RED BLOOD CELL
AChE ACTIVITY (U/mL) BY SAMPLE TIME

ICD No.	MREF No.		Sample Time Relative to Dosing GD					
			-65 min	-5 min	30 min	60 min	120 min	24 hr
-	PEG 540	N	44	40	44	43	44	15
		Mean	2.20	2.25	1.30	0.99	0.74	0.90
		SD*	0.30	0.34	0.62	0.63	0.55	0.60
1465	DP41-89	N	24	24	23	24	24	8
		Mean	2.21	2.24	2.11	1.72	1.23	1.37
		SD	0.35	0.38	0.39	0.55	0.55	0.74
1511	MA54-89	N	22	22	21	21	22	6
		Mean	2.33	2.25	2.13	1.98	1.78	2.05
		SD	0.26	0.32	0.36	0.36	0.33	0.30
1536	MS55-89	N	24	23	24	24	24	7
		Mean	2.25	2.31	1.38	0.97	0.68	0.95
		SD	0.30	0.43	0.48	0.37	0.30	0.56

*Standard deviation

TABLE 2. MREF TASK 89-03, PHASE 3 UNSTRESSED TSPs, IN VIVO, GD
STATISTICS FOR RELATIVE ACTIVITY (%) BY SAMPLE TIME

ICD No.	MREF No.		Sample Time Relative to Dosing GD			
			30 min	60 min	120 min	24 hr
-	PEG 540	N	44	43	44	15
		Mean	59.2	44.3	33.2	39.8
		SD*	26.7	25.6	22.2	24.3
1465	DP41-89	N	23	24	24	8
		Mean	97.3	78.7	56.4	59.9
		SD	16.1	22.6	23.7	31.2
1511	MA54-89	N	21	21	22	6
		Mean	91.0	84.9	76.9	83.6
		SD	13.5	14.1	14.5	20.8
1536	MS55-89	N	24	24	24	7
		Mean	61.0	43.3	30.4	43.2
		SD	18.9	16.2	14.2	25.5

*Standard deviation

TABLE 3. MREF TASK 89-03, PHASE 3, IN VIVO, GD RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 30 MIN

Order of Means	ICD No.	MREF No.		30 min	Grouping (Means with the same letter are equivalent)	
1	1465	DP41-89	N	23		
			Mean	97.3	A	
			SD*	16.1		
2	1511	MA54-89	N	21		
			Mean	91.0	A	
			SD	13.5		
3	1536	MS55-89	N	24		
			Mean	61.0		B
			SD	18.9		
4	-	PEG 540	N	44		
			Mean	59.2		B
			SD	26.7		

*Standard deviation

TABLE 4. MREF TASK 89-03, PHASE 3, IN VIVO, GD RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 60 MIN

Order of Means	ICD No.	MREF No.		60 min	Grouping (Means with the same letter are equivalent)
1	1511	MA54-89	N	21	
			Mean	84.9	A
			SD*	14.1	
2	1465	DP41-89	N	24	
			Mean	78.7	A
			SD	22.6	
3	-	PEG 540	N	43	
			Mean	44.3	B
			SD	25.6	
4	1536	MS55-89	N	24	
			Mean	43.3	B
			SD	16.2	

*Standard deviation

TABLE 5. MREF TASK 89-03, PHASE 3, IN VIVO, GD RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 120 MIN

Order of Means	ICD No.	MREF No.		120 min	Grouping (Means with the same letter are equivalent)		
1	1511	MA54-89	N	22			
			Mean	76.9	A		
			SD*	14.5			
2	1465	DP41-89	N	24			
			Mean	56.4		B	
			SD	23.7			
3	-	PEG 540	N	44			
			Mean	33.2			C
			SD	22.2			
4	1536	MS55-89	N	24			
			Mean	30.4			C
			SD	14.2			

*Standard deviation

TABLE 6. MREF TASK 89-03, PHASE 3, IN VIVO, GD
STATISTICS FOR MEAN RELATIVE ACTIVITY
ACROSS 30-, 60-, AND 120-MIN SAMPLES

Order of Means	ICD No.	MRCF No.			Grouping (Means with the same letter are equivalent)	
1	1511	MA54-89	N	20		
			Mean	0.836	A	
			SD*	0.109		
2	1465	DP41-89	N	23		
			Mean	0.767	A	
			SD	0.188		
3	-	PEG 540	N	43		
			Mean	0.453		B
			SD	0.239		
4	1536	MS55-89	N	24		
			Mean	0.449		B
			SD	0.144		

*Standard deviation

TABLE 7. MREF TASK 89-03, PHASE 3, IN VIVO EVALUATION OF TOPICAL SKIN PROTECTANTS FOR POSSIBLE ANTI-AChE EFFECTS INDEXED BY RED BLOOD CELL AChE ACTIVITY (U/mL) AT 65 AND 5 MIN BEFORE DOSING GD

ICD No.	MREF No.		Sample Time Relative to Dosing GD -65 min	-5 min	Paired Difference**
-	PEG 540	N	44	40	
		Mean	2.20	2.25	-0.01
		SD*	0.30	0.34	
1465	OP41-89	N	24	24	
		Mean	2.21	2.24	-0.03
		SD	0.35	0.38	
1511	MA54-89	N	22	22	
		Mean	2.33	2.25	0.08
		SD	0.26	0.32	
1536	MS55-89	N	24	23	
		Mean	2.25	2.31	-0.05
		SD	0.30	0.43	

*Standard deviation
**None were significant (P > 0.05, two-sided)

TABLE 3. MREF TASK 89-03, PHASE 3, IN VIVO EVALUATION OF TOPICAL SKIN PROTECTANTS FOR POSSIBLE LONG-TERM DELAY OF GD PENETRATION INDEXED BY RED BLOOD CELL AChE RELATIVE ACTIVITY (%) AT 120 MIN AND 24 HR AFTER DOSING GD

ICD No.	MREF No.		Sample Time Relative to Dosing GD 120 min	24 hr	Paired Difference
-	PEG 540	N	44	15	
		Mean	33.2	39.8	-7.8*
		SD**	22.2	24.3	
1465	DP41-89	N	24	8	
		Mean	56.4	59.9	1.4
		SD	23.7	31.2	
1511	MA54-89	N	22	6	
		Mean	76.9	83.6	-13.4*
		SD	14.5	20.8	
1536	MS55-89	N	24	7	
		Mean	30.4	43.2	-7.1
		SD	14.2	25.5	

*Significant ($P < 0.05$, two-sided) paired difference
**Standard deviation

TABLE 9. MREF TASK 89-03, PHASE 3, IN VIVO, VX STATISTICS FOR ABSOLUTE RED BLOOD CELL AChE ACTIVITY (U/mL) BY SAMPLE TIME

ICD No.	MREF No.		Sample Time Relative to Dosing VX -65 min	-5 min	30 min	60 min	120 min	24 hr
-	PEG 540	N (N dead)	46	44	46	46(1)	46(7)	12(8)
		Mean	2.18	2.25	0.98	0.48	0.24	0.22
		SD*	0.33	0.36	0.69	0.47	0.22	0.34
1465	DP41-89	N (N dead)	24	24	24	23(3)	24(12)	8(8)
		Mean	2.12	2.07	0.84	0.34	0.23	0.00
		SD	0.32	0.36	0.73	0.53	0.54	0.00
1511	MA54-89	N (N dead)	23	23	23	23	22(1)	7(1)
		Mean	2.29	2.19	2.05	1.67	1.47	1.33
		SD	0.35	0.41	0.51	0.64	0.74	0.89
1536	MS55-89	N (N dead)	22	21	22	22	22	7(5)
		Mean	2.22	2.32	1.81	1.16	0.56	0.22
		SD	0.42	0.49	0.54	0.71	0.50	0.39

*Standard deviation

TABLE 10. MREF TASK 89-03, PHASE 3 UNSTRESSED TSPs, IN VIVO, VX STATISTICS FOR RELATIVE ACTIVITY (%) BY SAMPLE TIME

ICD No.	MREF No.		Sample Time Relative to Dosing VX 30 min	60 min	120 min	24 hr
-	PEG 540	N	46	46	46	12
		Mean	44.6	22.3	10.9	11.1
		SD*	30.5	21.9	10.5	17.4
1465	DP41-89	N	24	23	24	8
		Mean	38.8	16.1	10.6	0.0
		SD	34.5	25.0	24.6	0.0
1511	MA54-89	N	23	23	22	7
		Mean	90.0	72.8	62.7	60.5
		SD	18.5	24.3	29.9	33.6
1536	MS55-89	N	22	22	22	7
		Mean	81.8	53.6	25.7	10.9
		SD	20.1	33.4	21.7	18.6

*Standard deviation

TABLE 11. MREF TASK 89-03, PHASE 3, IN VIVO, VX RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 30 MIN

Order of Means	ICD No.	MREF No.		30 min	Grouping (Means with the same letter are equivalent)	
1	1511	MA54-89	N	23		
			Mean	90.0	A	
			SD*	18.5		
2	1536	MS55-89	N	22		
			Mean	81.8	A	
			SD	20.1		
3	-	PEG 540	N	46		
			Mean	44.6		B
			SD	30.5		
4	1465	DP41-89	N	24		
			Mean	38.8		B
			SD	34.5		

*Standard deviation

TABLE 12. MREF TASK 89-03, PHASE 3, IN VIVO, VX RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 60 MIN

Order of Means	ICD No.	MREF No.		60 min	Grouping (Means with the same letter are equivalent)
1	1511	MA54-89	N	23	
			Mean	72.8	A
			SD*	24.3	
2	1536	MS55-89	N	22	
			Mean	53.6	A
			SD	33.4	
3	-	PEG 540	N	46	
			Mean	22.3	B
			SD	21.9	
4	1465	DP41-89	N	23	
			Mean	16.1	B
			SD	25.0	

*Standard deviation

TABLE 13. MREF TASK 89-03, PHASE 3, IN VIVO, VX RANKING OF TOPICAL SKIN PROTECTANTS INDEXED BY RELATIVE RED BLOOD CELL AChE ACTIVITY (%) AT 120 MIN

Order of Means	ICD No.	MREF No.		120 min	Grouping (Means with the same letter are equivalent)
1	1511	MA54-89	N Mean SD*	22 62.7 29.9	A
2	1536	MS55-89	N Mean SD	22 25.7 21.7	B
3	-	PEG 540	N Mean SD	46 10.9 10.5	B
4	1465	DP41-89	N Mean SD	24 10.6 24.6	B

*Standard deviation

TABLE 14. MREF TASK 89-03, PHASE 3, IN VIVO, VX STATISTICS FOR MEAN RELATIVE ACTIVITY ACROSS 30-, 60-, AND 120-MIN SAMPLES

Order of Means	ICD No.	MREF No.			Grouping (Means with the same letter are equivalent)
1	1511	MA54-89	N	22	
			Mean	0.749	A
			SD*	0.221	
2	1536	MS55-89	N	22	
			Mean	0.537	B
			SD	0.237	
3	-	PEG 540	N	46	
			Mean	0.260	C
			SD	0.199	
4	1465	DP41-89	N	23	
			Mean	0.223	C
			SD	0.253	

*Standard deviation

TABLE 15. MREF TASK 89-03, PHASE 3, IN VIVO EVALUATION OF TOPICAL SKIN PROTECTANTS FOR POSSIBLE ANTI-AChE EFFECTS INDEXED BY RED BLOOD BLOOD CELL AChE ACTIVITY (U/mL) AT 65 AND 5 MIN BEFORE DOSING VX

ICD No.	MREF No.		Sample Time Relative to Dosing: VX -65 min	-5 min	Paired Difference*
-	PEG 540	N	46	44	
		Mean	2.18	2.25	-0.08
		SD**	0.33	0.36	
1465	DP41-89	N	24	24	
		Mean	2.12	2.07	0.05
		SD	0.32	0.36	
1511	MA54-89	N	23	23	
		Mean	2.29	2.19	0.10
		SD	0.35	0.41	
1536	MS55-89	N	22	21	
		Mean	2.22	2.32	-0.07
		SD	0.42	0.49	

*None were significant ($P > 0.05$, two-sided)
**Standard deviation

TABLE 16. MREF TASK 89-03, PHASE 3, IN VIVO EVALUATION OF TOPICAL SKIN PROTECTANTS FOR POSSIBLE LONG-TERM DELAY OF VX PENETRATION INDEXED BY RED BLOOD CELL AChE RELATIVE ACTIVITY (%) AT 120 MIN AND 24 HR AFTER DOSING VX

ICD No.	MREF No.		Sample Time Relative to Dosing VX 120 min	24 hr	Paired Difference
-	PEG 540	N	46	12	
		Mean	10.9	11.1	-3.5
		SD*	10.5	17.4	
1465	DP41-89	N	24	8	
		Mean	10.6	0.0	0.4
		SD	24.6	0.0	
1511	MA54-89	N	22	7	
		Mean	62.7	60.5	-0.8
		SD	29.9	33.6	
1536	MS55-89	N	22	7	
		Mean	25.7	10.9	5.2**
		SD	21.7	18.6	

*Standard deviation
**Significant ($P < 0.05$, two-sided) paired difference

TABLE 17. MREF TASK 89-03, PHASE 3 IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS INDEXED BY HL LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS

ICD No.	MREF No.		Time After Dosing to Decontamination 1 hr	2 hr	4 hr
-	PEG 540	N	23	23	23
		Mean	253.0	371.5	361.8
		SD*	118.4	117.4	140.1
1465	DP41-89	N	23	23	23
		Mean	161.1	268.7	344.0
		SD	159.9	198.9	268.1
1511	MA54-89	N	24	24	24
		Mean	119.0	175.6	199.1
		SD	94.2	151.7	126.4
1536	MS55-89	N	24	24	24
		Mean	302.0	444.6	542.9
		SD	116.6	199.1	167.1

*Standard deviation

TABLE 18. MREF TASK 89-03, PHASE 3 IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS INDEXED BY HL LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

ICD No.	MREF No.		Time After Dosing to Decontamination 1 hr	2 hr	4 hr	Score**
-	PEG 540	N	23	23	23	23
		Mean	21.58	33.89	32.49	0.293
		SD*	9.42	18.29	16.48	0.136
1465	DP41-89	N	23	23	23	23
		Mean	12.10	20.87	26.45	0.198
		SD	10.45	12.68	17.92	0.127
1511	MA54-89	N	24	24	24	24
		Mean	7.99	11.90	13.63	0.112
		SD	6.59	10.08	8.45	0.065
1536	MS55-89	N	24	24	24	24
		Mean	22.13	31.65	38.42	0.307
		SD	11.51	15.71	14.30	0.124

*Standard deviation
**Mean of 1-, 2-, and 4-hr relative areas, expressed as a fraction

TABLE 19. MREF TASK 89-03, PHASE 3 ORDERING OF TOPICAL SKIN PROTECTANTS INDEXED BY HL LESION AREAS RELATIVE TO UNPROTECTED SITE (%) TIME TO DECONTAMINATION: 1 HR

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)	
1	1511	MA54-89	N	24		
			Mean	7.99	A	
			SD*	6.59		
2	1465	DP41-89	N	23		
			Mean	12.10	A	
			SD	10.45		
3	-	PEG 540	N	23		
			Mean	21.58		B
			SD	9.42		
4	1536	MS55-89	N	24		
			Mean	22.13		B
			SD	11.51		

*Standard deviation

TABLE 20. MREF TASK 89-03, PHASE 3 ORDERING OF TOPICAL SKIN PROTECTANTS INDEXED BY HL LESION AREAS RELATIVE TO UNPROTECTED SITE (%) TIME TO DECONTAMINATION: 2 HR

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)		
1	1511	MA54-89	N	24			
			Mean	11.90	A		
			SD*	10.08			
2	1465	DP41-89	N	23			
			Mean	20.87	A	B	
			SD	12.68			
3	1536	MS55-89	N	24			
			Mean	31.65		B	C
			SD	15.71			
4	-	PEG 540	N	23			
			Mean	33.89			C
			SD	18.29			

*Standard deviation

TABLE 21. MREF TASK 89-03, PHASE 3 ORDERING OF TOPICAL SKIN PROTECTANTS INDEXED BY HL LESION AREAS RELATIVE TO UNPROTECTED SITE (%) TIME TO DECONTAMINATION: 4 HR

Order of Means	ICD No.	MREF No.		Relative Area (%)	Grouping (Means with the same letter are equivalent)		
1	1511	MA54-89	N	24			
			Mean	13.63	A		
			SD*	8.45			
2	1465	DP41-89	N	23			
			Mean	26.45		B	
			SD	17.92			
3	-	PEG 540	N	23			
			Mean	32.49		B	C
			SD	16.48			
4	1536	MS55-89	N	24			
			Mean	38.42			C
			SD	14.30			

*Standard deviation

TABLE 22. MREF TASK 89-03, PHASE 3 ORDERING OF TOPICAL SKIN PROTECTANTS INDEXED BY MEAN OF HL LESION AREAS AT 1, 2, and 4 HR RELATIVE TO UNPROTECTED SITE, EXPRESSED AS A FRACTION

Order of Means	ICD No.	MREF No.		Relative Area	Grouping (Means with the same letter are equivalent)	
1	1511	MA54-89	N	24		
			Mean	0.112	A	
			SD*	0.065		
2	1465	DP41-89	N	23		
			Mean	0.198	A	
			SD	0.127		
3	-	PEG 540	N	23		
			Mean	0.293		B
			SD	0.136		
4	1536	MS55-89	N	2[illegible]		
			Mean	0.307		B
			SD	0.124		

*Standard deviation

APPENDIX E

The Efficacy of Lot 11JAN91BH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge

SPECIAL REPORT

Contract DAMD17-89-C-9050

on

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Dr. Garrett S. Dill
Dr. David W. Hobson
Mr. Thomas H. Snider

Battelle Columbus Operations
505 King Avenue
Columbus, Ohio 43201-2693

In conducting the research described in this report the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 86-23, revised 1985).

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SPECIAL REPORT

on

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

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Biostatistician

EXECUTIVE SUMMARY

This report describes the conduct of, and provides the results from, a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, "Test Up to Ten Candidate Topical Protectants", which was designed to evaluate the impact of treatment with a specific production lot of new candidate topical skin protectant, Multi-Shield® (ICD No. 1536, manufacturer's lot no. 11JAN91BH), to protect against the percutaneous toxicity of sulfur mustard (2,2'- dichlorodiethyl sulfide; HD) using a rabbit model. The nominal application thickness of the topical skin protectant (TSP) was 0.1 mm (using an application rate = 0.01 mL/cm^2) and HD applications were fixed at 1.0 µL per dose application site. HD toxicity was assessed following exposures for three different durations (1, 2, and 4 hr) prior to decontamination of each application site. In order to demonstrate the efficacy of ICD No. 1536 relative to a control TSP, dermal lesion areas from sites treated with ICD No. 1536 were calculated and were then statistically compared with pooled historical lesion area data from sites similarly treated with the control (which was a mixture of polyethylene glycols having an average molecular weight of 540 daltons; PEG 540).

Based on statistical comparisons of dermal lesion areas between ICD No. 1536 and the control TSP, the specific lot of ICD No. 1536 treatment demonstrated no significant ($P < 0.05$) protective effect against HD at any of the three exposure times relative to that of the control. These results differ from results previously obtained with another lot of ICD No. 1536 which indicated that ICD No. 1536 was significantly more effective than the control TSP against HD at all three exposure times.

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SPECIAL REPORT

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

1.0 INTRODUCTION

This report presents the results from a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, entitled "Test Up to Ten Candidate Topical Protectants". In this study, the efficacy of pretreating rabbit skin with a specific manufactured lot (lot no. 11JAN91BH) of Multi-Shield® (ICD No. 1536), a new topical skin protectant (TSP), was evaluated against percutaneous administered HD. The objective of this study was to determine whether a topically applied, 0.1 mm-thick, layer of this specific lot of ICD No. 1536 afforded increased protection against exposures to HD relative to the protection afforded by a control TSP. The evaluation was conducted using a rabbit model. Efficacy against HD exposure was determined from statistical tests based on the estimation of lesion area ratios (LARs) for each TSP-treated exposure site. LARs were calculated from the ratio of the HD-induced lesion area from each TSP-pretreated site relative to that of a non-pretreated, non-decontaminated control site on each rabbit. The study was performed in accordance with the phase one provisions for HD testing under Medical Research and Evaluation Facility (MREF) Protocol 58 (Attachment A).

2.0 METHODS

2.1 Test Materials

HD was obtained from the U.S. Army Medical Research and Development Command (USAMRDC). Chemical purity and appropriate identification were the responsibility of the USAMRDC. The HD used in these studies was identified as being from lot number U-6216-CTF-N-1. For quality control purposes, HD lots are periodically assayed for purity and stability at the MREF using an HD standard reference material supplied by the USAMRDC. Based on MREF gas

chromatographic analysis, HD from lot number U-6216-CTF-N-1 was found to be 82.3 percent pure at the time of the study.

The test TSP, identified as ICD No. 1536 (lot No. 11JAN91BH), was supplied by the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD). Chemical purity and appropriate identification were the responsibility of the USAMRICD.

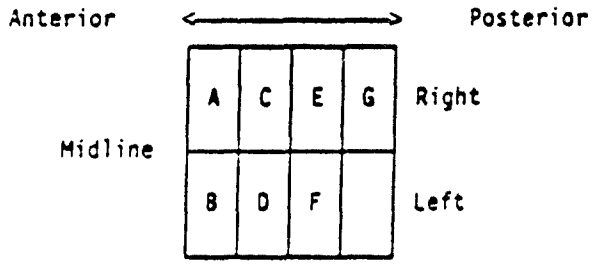
The control TSP was obtained from Union Carbide Corp., and consisted of a proprietary mixture of polyethylene glycol (Carbowax®; lot no. IS-403051) having an average molecular weight of 540 daltons (PEG 540).

2.2 Animal Model

Twenty-four, specific pathogen free, New Zealand White (albino), male rabbits weighing initially between 2.0 and 4.0 kg in weight were supplied by Hazleton Laboratories. Rabbits were chosen for this study because we have significant prior experience evaluating the percutaneous effects of HD and the application of candidate TSPs with this species. In accordance with the routine provisions of MREF Protocol 58 (Attachment A), the animals were randomly assigned to three weight-homogenized treatment groups of eight animals each and were prepared for treatment prior to study initiation.

2.3 Study Design

The methods detailed in MREF Protocol 58 (Appendix A) for phase one HD dosing only were followed in performing this study. The clipped dorsa of each rabbit was delineated into seven dosing areas of 2.5 cm by 5 cm which were designated as sites A through G as shown below:



The dosing area designated "G" was designated as an TSP- untreated control site. To each of three 2.5 x 5.0 cm dosing areas on all 24 animals, a 0.13-mL volume of ICD No. 1536 was applied from a 1 mL syringe (no needle) and spread to a uniform target thickness of 0.1 mm. The control TSP was similarly applied to each of three 2.5 x 5.0 cm dosing areas on eight of the 24 animals. Each TSP was allowed to remain undisturbed on the rabbit's back for approximately 60 min prior to HD challenge. Then, 1 μL of HD was applied to each of the TSP-treated test sites and the untreated control sites from a 1 μL gastight syringe equipped with a sharp-tipped needle. Care was exercised to ensure that the TSP layer was not mechanically disturbed in the dosing process. At the protocol-specified decontamination times (i.e., 1, 2, and 4 hr), each of the TSP application sites were decontaminated with a five-percent NaOCl solution followed by a distilled water rinse. The TSP-untreated control site "G" was similarly decontaminated immediately prior to the initiation of dermal lesion area evaluation (approximately 20 to 24 hr after HD application).

Twenty-four hours following HD-exposure, lesion lengths and widths were estimated from all HD dose sites, and absolute lesion areas were calculated. Absolute lesion area data from all TSP treated sites were expressed as LARs, i.e., ratios of the lesion area from TSP-protected, HD-exposed lesions to the TSP-unprotected, HD-exposed, non-decontaminated control lesion site on each rabbit (i.e. site "G"). Thus, a total of 24 LARs were estimated for ICD No. 1536 and eight for the control TSP. The control TSP LARs were statistically compared for compatibility with historical control data previously obtained under similar test conditions. The LARs for ICD No. 1,536 pretreatment versus those for control TSP treatment were then statistically compared using an unpaired t test with the alpha level set at 0.05 to evaluate the protective efficacy of ICD No. 1536 relative to that of the control TSP. To increase the statistical power of the comparison, the historical LARs for the control TSP were used for this comparison if the LARs from the current control TSP sample were found to be compatible with those of the historical data set.

3.0 RESULTS

Areas of lesions resulting from application of 1 μL of HD for individual animals are shown in Table 1. Univariate statistics for absolute HD lesion areas are shown in Table 2. Statistical comparison of ICD No. 1536 efficacy relative to the PEG 540 control and associated univariate statistics derived from HD LARs expressed as a percentage of the control dose site on the same rabbit are shown in Table 3. A summary of the historical LARs for the control TSP is shown in Table 4.

The number of rabbits (N = 148) and the mean LARs representing the PEG 540 quality control data base in Table 4 do not match those shown in Table 3 for PEG 540 (N = 124). The reason for this is that during the conduct of Task 89-03 three sets of eight rabbits were found to be outside critical limits and has to be replaced by additional sets (total = 24 rabbits) for a total of 124 rabbits in Table 3. However, all rabbits, even those in sets which exceeded the upper or lower control limits, were retained in the PEG 540 quality control data base (Table 4). One reason for this was that otherwise the critical limits would become closer to the mean as TSP screening proceeded, thereby moving the range for accepting a set of eight animals for a study. Table 4 indicates that LARs for the control TSP fell within the upper and lower critical limits as required for conducting a valid study.

Since the current data from the TSP control sites were found to be statistically compatible with the historical data, statistical comparisons could be made between the historical lesion area data for the control TSP and that of ICD No. 1536. As shown in Table 3, there was no statistically significant ($P < 0.05$) difference in the mean LAR estimated for ICD No. 1536 relative to that of the PEG 540 control thus indicating no significant difference in ICD No. 1536 efficacy relative to that of the control TSP. The statistical equivalence in the efficacies of ICD No. 1536 and PEG 540 to protect against HD exposure was evident at each of the exposure periods and for the overall mean performance index, referred to as "Score" in Table 3.

TABLE 1. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RESULTING FROM THREE EXPOSURE PERIODS: INDIVIDUAL RABBIT DATA

Site	G	A		C		E		B		D		F	
TSP/Time	none/24hr	PEG 540/1 hr		PEG 540/2 hr		PEG 540/4 hr		ICD 1536/1 hr		ICD 1536/2 hr		ICD 1536/4 hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
38065	198	75	38.1	92	46.4	126	63.5	95	48.0	170	85.7	179	90.5
37887	225	130	57.7	170	75.5	173	76.9	187	83.2	492	219.2	507	225.9
38589	174	112	64.7	107	61.5	99	57.0	107	61.5	194	111.8	123	70.6
38020	220	118	53.6	160	72.9	160	72.9	71	32.5	141	64.3	214	97.1
38279	328	134	40.9	179	54.5	174	52.9	121	36.8	188	57.4	143	43.5
38177	259	71	27.3	112	43.3	151	58.2	88	33.9	165	63.6	198	76.4
37799	240	82	34.0	71	29.7	106	44.1	60	25.2	113	47.1	92	38.2
38056	267	75	28.2	104	38.8	130	48.5	57	21.2	49	18.5	143	53.5
37795	368	.	.	.	.	.	.	104	28.2	179	48.7	132	35.9
38041	60	.	.	.	.	.	.	57	93.5	22	36.4	47	77.9
37769	78	.	.	.	.	.	.	42	54.5	28	36.4	0	0.0
37912	214	.	.	.	.	.	.	102	47.8	66	30.9	71	33.1
38072	503	.	.	.	.	.	.	151	30.0	181	36.1	478	95.0
37782	289	.	.	.	.	.	.	42	14.7	110	38.0	82	28.3
36633	289	.	.	.	.	.	.	31	10.9	60	20.9	173	59.8
38028	382	.	.	.	.	.	.	60	15.8	220	57.6	374	97.9
36687	225	.	.	.	.	.	.	55	24.5	88	39.2	85	37.8
38484	433	.	.	.	.	.	.	94	21.8	165	38.1	365	84.4
44706	487	.	.	.	.	.	.	50	10.3	209	42.9	374	76.8
38018	373	.	.	.	.	.	.	71	18.9	92	24.6	239	64.0
37709	487	.	.	.	.	.	.	181	37.3	286	58.7	358	73.5
37762	410	.	.	.	.	.	.	82	20.1	253	61.7	276	67.2
37707	164	.	.	.	.	.	.	42	25.8	42	25.8	106	64.6
38043	353	.	.	.	.	.	.	75	21.3	179	50.7	330	93.3

*Relative area, to G site.

TABLE 2. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS: SUMMARY STATISTICS

TSP		Time After Dosing to Decontamination		
		1 hr	2 hr	4 hr
ICD 1536	N	24	24	24
	MEAN	84.4	153.8	212.0
	SD	42.0	102.0	140.0
PEG 540	N	124	124	124
	MEAN	171.5	219.0	275.9
	SD	90.1	96.2	123.9

TABLE 3. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

TSP		Time After Dosing to Decontamination 1 hr	2 hr	4 hr	Score*
ICD 1539	N	24	24	24	24
	MEAN	34.07	54.76	70.22	0.5302
	STD	1.39	40.84	41.77	0.3011
PEG 540	N	124	124	124	124
	MEAN	38.92	50.47	63.40	0.5093
	STD	15.13	15.77	21.21	0.1560

*Mean of 1-, 2-, and 4-hr relative areas, expressed as a fraction.

TABLE 4. HISTORICAL PEG 540 CONTROL VERSUS CURRENT MEAN LESION AREA RATIOS (PERCENT RELATIVE TO NO-TSP CONTROL SITE)

	Time from Exposure to Decontamination					
	1 hr		2 hr		4 hr	
	Historical	Current	Historical	Current	Historical	Current
N	148	8	148	8	148	8
UCL	50.9		65.6		83.3	
Mean	37.9	43.1	49.7	52.8	63.0	59.2
LCL	24.9		33.8		42.7	

UCL = Upper critical limit, mean + 3 standard deviations
LCL = Lower critical limit, mean - 3 standard deviations

4.0 CONCLUSIONS

As shown above, ICD No. 1536 (Lot no. 11JAN91BH) demonstrated no significant ($P > 0.05$) protective efficacy in an HD challenge relative to that of the control TSP (PEG 540) It is noteworthy that this study was designed to demonstrate only the relative efficacy of ICD No. 1536 (lot no. 11JAN91BH) as compared to that of the control TSP and does not provide any information with regard to the absolute efficacy (relative to no TSP treatment) of the compound. As there are currently no data with which to address the absolute efficacy of either ICD No. 1536 or the control TSP, it may be desirable to consider the conduct of future studies to the assess the absolute efficacy of these compounds against HD exposure.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle Laboratory Notebook No. MREF - 220. Pre-study animal quarantine and observation records are on file at the MREF. All original data, as well as the original copy of this report will be maintained at the MREF until forwarded to the USAMRICD at the conclusion of the contract.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest academic degree of the principal contributors in this study are presented in the following list.

Name	Title	Degree
Garrett S. Dill	MREF Manager	D.V.M.
David W. Hobson	Study Director	Ph.D.
Thomas M. Snider	Biostatistician	B.S.
Peter L. Jepsen	Study Veterinarian	D.V.M.

APPENDIX F

The Efficacy of Lot 11JAN91BH of the Topical Skin Protectant, ICD No. 1536, Relative to No Protectant, Against a Sulfur Mustard Challenge

SPECIAL REPORT

Contract DAMD17-89-C-9050

on

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, RELATIVE TO NO PROTECTANT AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Dr. Garrett S. Dill
Dr. David W. Hobson
Mr. Thomas M. Snider

Battelle Columbus Operations
505 King Avenue
Columbus, Ohio 43201-2693

In conducting the research described in this report the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 86-23, revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

SPECIAL REPORT

on

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT,
ICD NO. 1536, RELATIVE TO NO PROTECTANT AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Garrett S. Dill, D.V.M. Date
Principal Investigator and
MREF Manager

David W. Hooson, Ph.D., D.A.B.T. Date
Study Director

Thomas S. Snider, B.S., D.A.B.T. Date
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Based on statistical comparisons of dermal lesion areas between ICD No. 1536 and no TSP, the specific lot of ICD No. 1536 treatment demonstrated a significant ($P < 0.05$) protective effect against HD at the 5-and 30-min but not at the 60-min exposure times.

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SPECIAL REPORT

THE EFFICACY OF LOT 11JAN91BH OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, RELATIVE TO NO PROTECTANT AGAINST A SULFUR MUSTARD CHALLENGE

1.0 INTRODUCTION

This report presents the results from a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, entitled "Test Up to Ten Candidate Topical Protectants". In this study, the efficacy of pretreating rabbit skin with a specific manufactured lot (lot no. 11JAN91BH) of Multi-Shield® (ICD No. 1536), a new topical skin protectant (TSP), was evaluated against percutaneous administered HD. The objective of this study was to determine whether a topically applied, 0.1 mm-thick, layer of this specific lot of ICD No. 1536 afforded increased protection against exposures to HD relative to unprotected dose sites. The evaluation was conducted using a rabbit model. Efficacy against HD exposure was determined from statistical tests based on the estimation of lesion area ratios (LARs) for each exposure site. LARs were calculated from the ratio of the HD-induced lesion area from each HD dose site relative to that of a non-pretreated, 24-hr decontaminated control site on each rabbit. The study was performed in accordance with the phase one provisions for HD testing under Medical Research and Evaluation Facility (MREF) Protocol 58 (Attachment A).

2.0 METHODS

2.1 Test Materials

HD was obtained from the U.S. Army Medical Research and Development Command (USAMRDC). Chemical purity and appropriate identification were the responsibility of the USAMRDC. The HD used in these studies was identified as being from lot number U-6216-CTF-N-1. For quality control purposes, HD lots are periodically assayed for purity and stability at the MREF using an HD standard reference material supplied by the USAMRDC. Based on MREF gas chromatographic analysis, HD from lot number U-6216-CTF-N-1 was found to be 82.3 percent pure at the time of the study.

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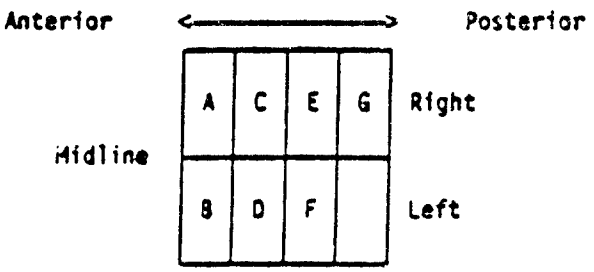
2.2 Animal Model

Twenty-four, specific pathogen free, New Zealand White (albino), male rabbits weighing initially between 2.0 and 4.0 kg in weight were supplied by Hazleton Laboratories. Rabbits were chosen for this study because we have significant prior experience evaluating the percutaneous effects of HD and the application of candidate TSPs with this species. In accordance with the routine provisions of MREF Protocol 58 (Attachment A), the animals were randomly assigned to three weight-homogenized treatment groups of eight animals each and were prepared for treatment prior to study initiation.

2.3 Study Design

The methods detailed in MREF Protocol 58 (Appendix A) for phase one HD dosing only were followed in performing this study. The clipped dorsa of each rabbit was delineated into seven dosing areas of 2.5 cm by 5 cm which were designated as sites A through G as shown below:



The dosing area designated "G" was designated as a TSP-untreated, 24-hr decontaminated control site. To each of three 2.5 x 5.0 cm dosing areas on all 24 animals, a 0.13-mL volume of ICD No. 1536 was applied from a 1 mL syringe (no needle) and spread to a uniform target thickness of 0.1 mm. TSP was allowed to remain undisturbed on the rabbit's back for approximately

60 min prior to HD challenge. Then, 1 μL of HD was applied to each of the test sites and the untreated control sites from a 1 μL gastight syringe equipped with a sharp-tipped needle. Care was exercised to ensure that the TSP layer was not mechanically disturbed in the dosing process. At the decontamination times specified for this study, (i.e., 5, 30, and 60 min), each of the test sites (A through F) was decontaminated with a five-percent NaOCl solution followed by a distilled water rinse. The TSP-untreated control site "G" was similarly decontaminated immediately prior to the initiation of dermal lesion area evaluation (approximately 20 to 24 hr after HD application).

Twenty-four hours following HD-exposure, lesion lengths and widths were estimated from all HD dose sites, and absolute lesion areas were calculated. Absolute lesion area data from all TSP-treated sites were expressed as LARs, i.e., ratios of the lesion area from test HD-exposed lesions to the TSP-untreated, HD-exposed, 24-hr decontaminated control lesion site on each rabbit (i.e. site "G"). Thus, a total of 24 LARs each were estimated for ICD No. 1536 and contralateral sites. Individual animal "G" site lesion areas were screened for outliers according to whether they were within the range between the historical mean plus or minus three standard deviations. The LARs for ICD No. 1536 pretreatment versus those for no TSP treatment were then statistically compared using a paired (by animal) t test with the alpha level set at 0.05 to evaluate the protective efficacy of ICD No. 1536. The paired t test was used to increase the statistical power of the comparison relative to the unpaired t test.

3.0 RESULTS

Areas of lesions resulting from application of 1 μL of HD for individual animals are shown in Table 1. Four rabbits presented TSP-untreated, 24-hr decontaminated control G sites with area estimates greater than the historical mean plus three standard deviations. Thus, data for those four rabbits were removed from the data base. Univariate statistics for absolute HD lesion areas for the remaining 20 rabbits are shown in Table 2. Statistical comparison of ICD No. 1536 efficacy relative to no TSP and associated univariate statistics derived from HD LARs expressed as a percentage of the control dose site on the same rabbit are shown in Table 3.

Rabbit-paired differences between LAR data for ICD No. 1536 and the contralateral untreated site decontaminated at the same time were calculated, and a paired t test was applied. The paired t test was also applied to contralateral differences in the "Score," i.e., the mean LAR across exposure periods. As shown in Table 3, there was a statistically significant ($P < 0.05$) paired difference in the LARs estimated for ICD No. 1536 relative to that of the contralateral site for 5 and 30 min HD exposure periods, but not at 60 min. The contralateral Score difference was also significant ($P < 0.05$), thus indicating overall efficacy of ICD No. 1536.

TABLE 1. IN VIVO ASSESSMENT OF ICD NO. 1536 INDEXED BY NO LESION AREAS RESULTING FROM THREE EXPOSURE PERIODS: INDIVIDUAL RABBIT DATA

Site	G	A		C		E		B		D		F	
ISP/Time	None/24 hr	None/5 min		None/30 min		None/60 min		ICD 1536/5 min		ICD 1536/30 min		ICD 1536/60 min	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
37746	672	88	13.1	255	38.0	276	41.1	92	13.7	61	9.1	107	15.9
38102	664	47	7.1	207	31.2	601	90.4	16	2.5	47	7.1	164	24.7
38112	639	88	13.8	289	45.2	259	40.5	39	6.1	66	10.3	99	15.5
38221	536	88	16.4	245	45.7	302	56.3	60	11.3	110	20.5	160	29.9
38091	401	66	16.5	179	44.7	242	60.4	60	15.1	179	44.7	259	64.7
38239	402	57	14.1	164	40.8	226	56.3	66	16.4	132	32.8	134	33.2
37713	528	71	13.5	120	22.8	302	57.1	25	4.8	75	14.3	132	25.0
37733	808	35	4.4	107	13.2	194	24.0	28	3.5	69	8.6	112	13.9
39177	537	66	12.3	157	29.2	209	38.9	38	7.2	160	29.8	283	52.6
38017	537	28	5.3	194	36.1	217	40.4	16	2.9	60	11.3	120	22.4
38238	484	75	15.6	201	41.6	339	70.1	33	6.8	224	46.3	198	40.9
36863	2136**	71	3.3	134	6.3	264	12.4	69	3.2	163	7.6	174	8.1
44705	628	110	17.5	347	55.3	330	52.5	57	9.0	143	22.8	165	26.3
37740	393	121	30.8	267	68.0	361	92.0	47	12.0	44	11.2	85	21.6
37741	462	75	16.3	198	42.9	294	63.6	69	15.0	127	27.6	160	34.7
38040	462	71	15.3	160	34.7	204	44.2	31	6.8	94	20.4	259	56.1
38134	966**	64	6.6	373	38.6	511	52.9	75	7.8	179	18.5	396	41.0
38265	271	71	26.1	147	54.2	132	48.7	7	2.6	85	31.3	130	47.8
39151	1095**	92	8.4	353	32.3	330	30.1	82	7.5	561	51.2	170	15.5
37744	597	141	23.7	264	44.2	438	73.4	71	11.8	130	21.7	220	36.8
38010	1156**	86	7.5	204	17.7	471	40.8	44	3.8	112	9.7	170	14.7
39138	276	132	47.7	212	76.7	236	85.2	69	25.0	85	30.7	236	85.2
38084	653	69	10.6	153	23.4	313	48.0	86	13.2	143	21.9	432	66.1
38299	596	85	14.2	102	17.1	226	37.9	50	8.4	121	20.3	884	148.2

*Relative area, to G site.
**Greater than historical mean plus three standard deviations.

TABLE 2. IN VIVO ASSESSMENT OF ICD NO. 1536 INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS: SUMMARY STATISTICS

		Time After Dosing to Decontamination		
TSP		5 min	30 min	60 min
ICD 1536	N	20	20	20
	MEAN	48.0	107.3	217.0
	SD	23.9	47.7	177.3
Nothing	N	20	20	20
	MEAN	79.2	198.4	285.1
	SD	29.3	64.2	101.6

TABLE 3. IN VIVO ASSESSMENT OF ICD NO. 1536 INDEXED BY MD LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

		Time After Dosing to Decontamination			
TSP		5 min	30 min	60 min	Score*
ICD 1539	N	20	20	20	20
	MEAN	9.7	22.1	43.1	0.250
	SD	5.7	11.4	31.4	0.132
Nothing	N	20	20	20	20
	MEAN	16.7	40.3	56.1	0.377
	SD	9.7	15.8	18.5	0.129
Paired Difference	N	20	20	20	20
	MEAN	7.0	18.1	13.0	0.127
	SD	7.5	16.8	37.3	0.170
Paired T		4.185	4.814	1.556	3.346
Probability > |T|		0.0005	0.0001	0.1361	0.0034

*Mean of 5-, 30-, and 60-min relative areas, expressed as a fraction.

4.0 CONCLUSIONS

As shown above, ICD No. 1536 (Lot no. 11JAN91BH) demonstrated significant ($P < 0.05$) protective efficacy against an HD challenge relative to nothing at the 5- and 30- min exposure periods. There was no efficacy demonstrated at the 60-min exposure period. The overall index of efficacy, called "Score" in Table 3, indicated a statistically significant difference between sites pretreated with ICD No. 1536 and nothing. However, the low magnitude of the "Score" difference (approximately 13 percent) indicates that increasing the duration of the HD exposure period tends to adversely affect the efficacy of ICD No. 1536 against an HD challenge.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle Laboratory Notebook No. MREF - 220. Pre-study animal quarantine and observation records are on file at the MREF. All original data, as well as the original copy of this report will be maintained at the MREF until forwarded to the USAMRICD at the conclusion of the contract.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest academic degree of the principal contributors in this study are presented in the following list.

Name	Title	Degree
Garrett S. Dill	MREF Manager	D.V.M.
David W. Hobson	Study Director	Ph.D.
Thomas H. Snider	Biostatistician	B.S.
Peter L. Jepsen	Study Veterinarian	D.V.M.

APPENDIX G

The Efficacy of Lot 17JAN91BH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge

SPECIAL REPORT

Contract DAMD17-89-C-9050

on

THE EFFICACY OF LOT 17JAN91B OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Dr. Garrett S. Dill
Dr. David W. Hobson
Mr. Thomas M. Snider

Battelle Columbus Operations
505 King Avenue
Columbus, Ohio 43201-2693

In conducting the research described in this report the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 86-23, revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

SPECIAL REPORT

on

THE EFFICACY OF LOT 17JAN91B OF THE TOPICAL SKIN PROTECTANT,
ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Garrett S. Gill, D.V.M. Date
Principal Investigator and
MREF Manager

David W. Hobson, Ph.D., D.A.B.T. 1/22/91 Date
Study Director

Thomas S. Snider 1/22/91
Thomas S. Snider, B.S., D.A.B.T. Date
Biostatistician

EXECUTIVE SUMMARY

This report describes the conduct of, and provides the results from, a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, "Test Up to Ten Candidate Topical Protectants", which was designed to evaluate the impact of treatment with a specific production lot of new candidate topical skin pro[illegible]Multi-Shield® (ICD No. 1536, manufacturer's lot no. 17JAN91[illegible]tect against the percutaneous toxicity of sulfur mustard (2,2'- dichlo[illegible]sulfide; HD) using a rabbit model. The nominal application thickness of topical skin protectant (TSP) was 0.1 mm (using an application rate of [illegible] g/cm^2), and HD applications were fixed at 1.0 µL per dose application site. Toxicity was assessed following exposures for three different durations[illegible] and 4 hr) prior to decontamination of each application site. In order to demonstrate the efficacy of ICD No. 1536 relative to a control TSP, dermal lesion areas from sites treated with ICD No. 1536 were calculated and were then statistically compared with pooled historical lesion area data from sites similarly treated with the control (which was a mixture of polyethylene glycols having an average molecular weight of 540 daltons; PEG 540). Results from ICD No. 1536 were also compared with data from the remaining 16 rabbits at sites not pretreated with TSP, but dosed with HD and decontaminated at the same times as ICD No. 1536 at contralateral sites.

Based on statistical comparisons of dermal lesion areas between ICD No. 1536 and the control TSP, lot 17JAN91B of ICD No. 1536 treatment demonstrated no significant ($P > 0.05$, adjusted for multiple comparisons) protective effect against HD at any of the three exposure times relative to no TSP. Relative to PEG 540, lot 17JAN91B of ICD No. 1536 treatment was equivocal at 1- and 2-hr exposures, but was significantly ($P < 0.05$) less protective against HD at the 4-hr exposure period.

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SPECIAL REPORT

THE EFFICACY OF LOT 17JAN91B OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

1.0 INTRODUCTION

This report presents the results from a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, entitled "Test Up to Ten Candidate Topical Protectants". In this study, the efficacy of pretreating rabbit skin with a specific manufactured lot (lot no. 17JAN91B) of Multi-Shield® (ICD No. 1536), a new topical skin protectant (TSP), was evaluated against percutaneous administered HD. The objective of this study was to determine whether a topically applied, 0.1 mm-thick, layer of this specific lot of ICD No. 1536 afforded increased protection against exposures to HD relative to both no TSP and the protection afforded by a control TSP. The evaluation was conducted using a rabbit model. Efficacy against HD exposure was determined from statistical tests based on the estimation of lesion area ratios (LARs) for each TSP-treated exposure site. LARs were calculated from the ratio of the HD-induced lesion area from each TSP-pretreated site relative to that of a non-pretreated, 24 hr-decontaminated control site on each rabbit. The study was performed in accordance with the phase one provisions for HD testing under Medical Research and Evaluation Facility (MREF) Protocol 58 (Attachment A).

2.0 METHODS

2.1 Test Materials

HD was obtained from the U.S. Army Medical Research and Development Command (USAMRDC). Chemical purity and appropriate identification were the responsibility of the USAMRDC. The HD used in these studies was identified as being from lot number U-6216-CTF-N-1. For quality control purposes, HD lots are periodically assayed for purity and stability at the MREF using an HD standard reference material supplied by the USAMRDC. Based on MREF gas

chromatographic analysis, HD from lot number U-6216-CTF-N-1 was found to be 82.3 percent pure at the time of the study.

The test TSP, identified as ICD No. 1536 (lot No. 17JAN91B), was supplied by the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD). Chemical purity and appropriate identification were the responsibility of the USAMRICD.

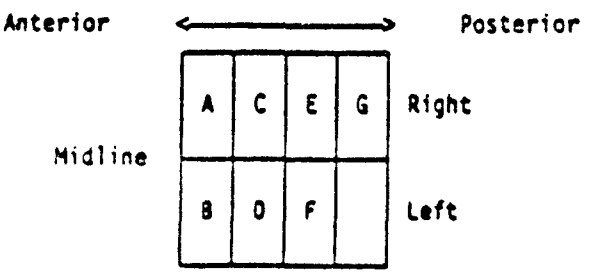
The control TSP was obtained from Union Carbide Corp., and consisted of a proprietary mixture of polyethylene glycol (Carbowax®; lot no. IS-403051) having an average molecular weight of 540 daltons (PEG 540).

2.2 Animal Model

Twenty-four, specific pathogen free, New Zealand White (albino), male rabbits weighing initially between 2.0 and 4.0 kg in weight were supplied by Hazleton Laboratories. Rabbits were chosen for this study because we have significant prior experience evaluating the percutaneous effects of HD and the application of candidate TSPs with this species. In accordance with the routine provisions of MREF Protocol 58 (Attachment A), the animals were randomly assigned to three weight-homogenized treatment groups of eight animals each and were prepared for treatment prior to study initiation.

2.3 Study Design

The methods detailed in MREF Protocol 58 (Appendix A) for phase one HD dosing only were followed in performing this study. The clipped dorsa of each rabbit was delineated into seven dosing areas of 2.5 cm by 5 cm which were designated as sites A through G as shown below:



The dosing area designated "G" was designated as a TSP-untreated control site. To each of three 2.5 x 5.0 cm dosing areas (B, D, and F) on all 24 animals, a 0.13-mL volume of ICD No. 1536 was applied from a 1 mL syringe (no needle) and spread to a uniform target thickness of 0.1 mm. The control TSP was similarly applied to each of three 2.5 x 5.0 cm dosing areas (A, C, and E) on eight of the 24 animals. Sites Ac, C, and e on the 16 rabbits were untreated. Each TSP was allowed to remain undisturbed on the rabbit's back for approximately 60 min prior to HD challenge. Then, 1 µL of HD was applied to each of the TSP-treated test sites and the untreated control sites from a 1 µL gastight syringe equipped with a sharp-tipped needle. Care was exercised to ensure that the TSP layer was not mechanically disturbed in the dosing process. At the protocol-specified decontamination times (i.e., 1, 2, and 4 hr), each of the TSP application sites was decontaminated with a five percent NaOCl solution followed by a distilled water rinse. The TSP-untreated control site "G" was similarly decontaminated immediately prior to the initiation of dermal lesion area evaluation (approximately 20 to 24 hr after HD application).

Twenty-four hours following HD-exposure, lesion lengths and widths were estimated from all HD dose sites, and absolute lesion areas were calculated. Absolute lesion area data from all TSP treated sites were expressed as LARs, i.e., ratios of the lesion area from HD-exposed test sites to the TSP-unprotected, HD-exposed, 24 hr-decontaminated control lesion site on each rabbit (i.e. site "G"). Thus, 24 LARs were estimated for ICD No. 1536, eight for the control TSP, and 16 for no-TSP sites at each exposure period. The control TSP LARs were statistically compared for compatibility with historical control data previously obtained under similar test conditions. The LARs for ICD No. 1536 pretreatment versus those for control TSP treatment were then statistically compared using an unpaired t test with the overall alpha level set at 0.05 to contrast the protective efficacy of ICD No. 1536 relative to that of the control TSP and no TSP. To increase the statistical power of the comparison, the historical LARs for the control TSP were used for this comparison if the LARs from the current control TSP sample were found to be compatible with those of the historical data set.

3.0 RESULTS

Areas of lesions resulting from application of 1 μL of HD for individual animals are shown in Table 1. Univariate statistics for absolute HD lesion areas are shown in Table 2. Statistical comparison of ICD No. 1536 efficacy relative to the PEG 540 control and no TSP, and associated univariate statistics derived from HD LARs expressed as a percentage of the control dose site on the same rabbit are shown in Table 3. A summary of the historical LARs for the control TSP is shown in Table 4.

The number of rabbits (N = 156) and the mean LARs representing the PEG 540 quality control data base in Table 4 do not match those shown in Table 3 for PEG 540 (N = 132). The reason for this is that during the conduct of Task 89-03 three sets of eight rabbits were found to be outside critical limits and has to be replaced by additional sets (total = 24 rabbits) for a total of 124 rabbits in Table 3. However, all rabbits, even those in sets which exceeded the upper or lower contr[illegible]its, were retained in the PEG 540 quality control data base (Table 4). Ou[illegible] for this was that otherwise the critical limits would become closer to the mean as TSP screening proceeded, thereby moving the range for accepting a set of eight animals for a study. Table 4 indicates that LARs for the control TSP fell within the upper and lower critical limits as required for conducting a valid study.

Since the current data from the TSP control sites were found to be statistically compatible with the historical data, statistical comparisons could be made between the historical lesion area data for the control TSP and that of ICD No. 1536. As shown in Table 3 at the 1- and 2-hr exposure periods, there was no statistically significant ($P > 0.05$) difference in the mean LAR estimated for ICD No. 1536 relative to that of the PEG 540 control, thus indicating no significant difference in ICD No. 1536 efficacy relative to that of the control TSP. At the 4-hr exposure period, pretreating with ICD No. 1536 was equivocal with no TSP application and significantly less effective than PEG 540. The statistical equivalence in the efficacies of ICD No. 1536, PEG 540, with no TSP against HD exposure was evident at the overall mean performance index, referred to as "Score" in Table 3.

TABLE 1. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RESULTING FROM THREE EXPOSURE PERIODS: INDIVIDUAL RABBIT DATA

Site	C	A		C		E		B		D		F	
TSP/Time	none/24hr	PEG 540/1 hr		PEG 540/2 hr		PEG 540/4 hr		ICD 1536/1 hr		ICD 1536/2 hr		ICD 1536/4 hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
40071	373	104	27.8	78	20.8	236	63.2	214	57.3	245	65.7	385	103.2
39280	470	110	23.4	130	27.6	151	32.1	31	6.7	78	16.6	92	19.6
38123	198	66	33.3	77	38.9	94	47.6	47	23.8	101	50.8	163	82.5
38406	448	132	29.5	226	50.5	339	75.8	71	15.8	302	67.4	393	87.7
40181	226	94	41.7	86	38.2	132	58.3	63	27.8	164	72.6	283	125.0
39537	492	212	43.1	247	50.2	302	61.2	187	38.0	412	83.7	440	89.3
38980	553	188	34.1	251	45.5	418	75.6	79	14.2	297	53.7	440	79.5
39507	471	236	50.0	325	69.0	377	80.0	251	53.3	214	45.5	424	90.0
39116	429	174	40.5	187	43.6	254	59.3	118	27.5	110	25.6	253	59.0
37760	622	297	47.7	271	43.6	242	38.9	227	36.5	194	31.2	334	53.7
38176	47	71	150.0	121	255.7	86	183.3	85	180.0	123	260.0	141	300.0
38128	176	60	34.4	75	42.9	112	63.8	24	13.4	31	17.9	49	28.1
39102	330	101	30.5	170	51.4	226	68.6	242	73.3	173	52.4	275	83.3
39255	194	52	26.7	88	45.3	113	58.3	42	21.9	49	25.5	64	32.8
39225	433	132	30.5	220	50.8	289	66.8	92	21.2	160	37.0	198	45.7
39234	343	113	33.0	200	58.4	294	85.6	194	56.5	253	73.7	360	105.0
39186	403	60	15.0	99	24.6	174	43.1	123	30.4	160	39.8	194	48.1
38911	339	77	22.7	164	48.4	253	74.5	60	17.8	110	32.4	163	48.1
37739	427	119	27.9	225	52.6	286	66.9	49	11.6	110	25.7	297	69.5
43711	283	253	89.4	259	91.7	302	106.7	138	48.9	170	60.0	170	60.0
38159	235	118	50.2	138	58.9	220	93.6	66	28.1	130	55.2	106	45.2
37756	224	99	44.2	160	71.6	147	65.6	47	21.1	110	49.1	153	68.4
39419	226	71	31.3	141	62.5	212	93.8	22	9.7	147	64.9	225	99.3
42160	360	126	34.9	204	56.6	264	73.2	113	31.4	113	31.4	259	71.9

*Relative area, to C site.

TABLE 2. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS: SUMMARY STATISTICS

		Time After Dosing to Decontamination		
TSP		1 hr	2 hr	4 hr
ICD 1536	N	24	24	24
	MEAN	107.7	164.8	244.2
	SD	73.3	87.5	120.4
None	N	16	16	16
	MEAN	120.2	170.1	217.1
	SD	69.0	58.4	70.5
PEG 540	N	132	132	132
	MEAN	169.7	216.5	274.7
	SD	88.7	96.3	123.4

TABLE 3. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

TSP		Time After Dosing to Decontamination 1 hr	2 hr	4 hr	Score**
MS70-91	N	24	24	24	24
	MEAN	36.1 A	55.7 A	79.0 A	0.569 A
	SD	35.1	47.4	54.0	0.441
None	N	16	16	16	16
	MEAN	44.3 A	66.2 A	77.3 A B	0.627 A
	SD	32.7	52.8	33.3	0.386
PEG 540	N	132	132	132	132
	MEAN	38.7 A	50.0 A	63.3 B	0.507 A
	SD	14.8	15.8	20.9	0.154

* Groups having statistically indistinguishable means are indicated with the same letter at each exposure period and Score.
** Mean of 1-, 2-, and 4-hr relative areas, expressed as a fraction.

TABLE 4. HISTORICAL PEG 540 CONTROL VERSUS CURRENT MEAN LESION AREA RATIOS (PERCENT RELATIVE TO NO-TSP CONTROL SITE)

	Time from Exposure to Decontamination					
	1 hr		2 hr		4 hr	
	Historical	Current	Historical	Current	Historical	Current
N	156	8	156	8	156	8
UCL	50.6		65.2		83.1	
Mean	37.8	35.4	49.3	42.6	62.9	61.[illegible]
LCL	25.0		33.4		42.7	

UCL = Upper critical limit, mean + 3 standard deviations
LCL = Lower critical limit, mean - 3 standard deviations

4.0 CONCLUSIONS

ICD No. 1535 (Lot no. 17JAN918) demonstrated neither significant ($P > 0.05$) protective efficacy against an HD challenge relative to that of the control TSP (PEG 540) nor absolute efficacy relative to no TSP.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle Laboratory Notebook No. MREF - 220. Pre-study animal quarantine and observation records are on file at the MREF. All original data, as well as the original copy of this report will be maintained at the MREF until forwarded to the USAMRICD at the conclusion of the contract.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest academic degree of the principal contributors in this study are presented in the following list.

Name	Title	Degree
Garrett S. Dill	MREF Manager	D.V.M.
David W. Hobson	Study Director	Ph.D.
Thomas H. Snider	Biostatistician	B.S.
Peter L. Jepsen	Study Veterinarian	D.V.M.

APPENDIX H

The Efficacy of Lot 5255 of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge

SPECIAL REPORT

Contract DAMD17-89-C-9050

on

THE EFFICACY OF LOT 5256 OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

Dr. Garrett S. Dill
Dr. David W. Hobson
Mr. Thomas H. Saider

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505 King Avenue
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SPECIAL REPORT

on

THE EFFICACY OF LOT 5256 OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

January, 1991

David W. Hobson for 1/24/91
Garrett S. Dill, D.V.M. Date
Principal Investigator and
MREF Manager

David W. Hobson 1/24/91
David W. Hobson, Ph.D., D.A.B.T. Date
Study Director

Thomas S. Snider 1/24/91
Thomas S. Snider, B.S., D.A.B.T. Date
Biostatistician

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EXECUTIVE SUMMARY

This report describes the conduct of, and provides the results from, a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, "Test Up to Ten Candidate Topical Protectants", which was designed to re-evaluate the impact of treatment with a specific production lot of a candidate topical skin protectant, Multi-Shield® (ICD No. 1536, manufacturer's Lot No. 5256), to protect against the percutaneous toxicity of sulfur mustard (2,2'- dichlorodiethyl sulfide; HD) using a rabbit model. This lot number of the candidate skin protectant was previously tested at the MREF, and the results were reported in a letter report to U.S. Army Medical Research and Development Command (USAMRDC) dated 17 August 1990. The purpose of the present study was to reassess the efficacy of ICD No. 1536, Lot No. 5256, relative to a control topical skin protectant (TSP) and establish its absolute efficacy relative to no TSP. The nominal application thickness of the TSP was 0.1 cm (using an application rate = 0.01 mL/cm^2), and HD applications were fixed at 1.0 µL per dose application site. HD toxicity was assessed following exposures for three different durations (1, 2, and 4 hr) prior to decontamination of each application site. In order to demonstrate the efficacy of ICD No. 1536 relative to a control TSP, dermal lesion areas from sites treated with ICD No. 1536 were calculated and were then statistically compared with pooled historical lesion area data from sites similarly treated with the control (which was a mixture of polyethylene glycols having an average molecular weight of 540 daltons; PEG 540). Results from ICD No. 1536 were also compared with data from a pool of the remaining 16 rabbits with 16 rabbits from a previous study pretreated with no TSP, but dosed with HD and decontaminated at the same times as ICD No. 1536 at contralateral sites.

Based on statistical comparisons between historical and current results of dermal lesion area ratios obtained for ICD No. 1536, (Lot No. 5256) there was no significant difference in the protective efficacy noted at any of the HD exposure periods. Relative to both no TSP and PEG 540, both sets of results for Lot 5256 of ICD No. 1536 treatment demonstrated a significant ($P < 0.05$, adjusted for multiple comparisons) protective effect against HD at all of the three exposure times.

SPECIAL REPORT

THE EFFICACY OF LOT 5256 OF THE TOPICAL SKIN PROTECTANT, ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

1.0 INTRODUCTION

This report presents the results from a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, entitled "Test Up to Ten Candidate Topical Protectants". In this study, the efficacy of pretreating rabbit skin with a specific manufactured lot (Lot No. 5256) of Multi-Shield® (ICD No. 1536), a new topical skin protectant (TSP), was evaluated against percutaneous by administered HD. The objectives of this study were to determine whether current results from a topically applied, 0.1 mm-thick, layer of this specific lot of ICD No. 1536 were consistent with those from a previous test, and whether increased protection against exposures to HD relative to both no TSP and the protection afforded by a control TSP was evident following ICD No. 1536 application. The evaluation was conducted using a rabbit model. Efficacy against HD exposure was determined from statistical tests based on the estimation of lesion area ratios (LARs) for each test site. LARs were calculated from the ratio of the HD-induced lesion area from each test site relative to that of a non-pretreated, 24 hr-decontaminated control site on each rabbit. The study was performed in accordance with the phase one provisions for HD testing under Medical Research and Evaluation Facility (MREF) Protocol 58 (Attachment A).

2.0 METHODS

2.1 Test Materials

HD was obtained from the U.S. Army Medical Research and Development Command (USAMRDC). Chemical purity and appropriate identification were the responsibility of the USAMRDC. The HD used in these studies was identified as being from Lot No. U-6216-CTF-N-1. For quality control purposes, HD lots are periodically assayed for purity and stability at the MREF using an HD standard reference material supplied by the USAMRDC. Based on MREF gas chromatographic

analysis, HD from Lot No. U-6216-CTF-N-1 was found to be 82.3 percent pure at the time of the study.

The test TSP, identified as ICD No. 1536 (Lot No. 5256), was supplied by the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD). Chemical purity and appropriate identification were the responsibility of the USAMRICD.

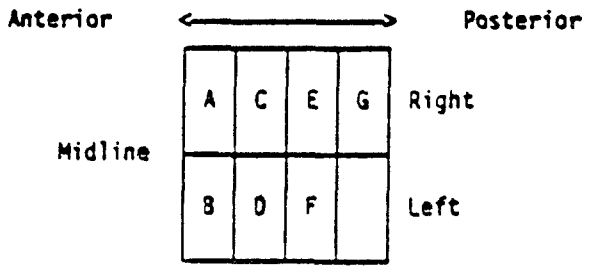
The control TSP was obtained from Union Carbide Corp., and consisted of a proprietary mixture of polyethylene glycol (Carbowax®; Lot No. IS-403051) having an average molecular weight of 540 daltons (PEG 540).

2.2 Animal Model

Twenty-four, specific pathogen free, New Zealand White (albino), male rabbits weighing initially between 2.0 and 4.0 kg in weight were supplied by Hazleton Laboratories. Rabbits were chosen for this study because we have significant prior experience evaluating the percutaneous effects of HD and the application of candidate TSPs with this species. In accordance with the routine provisions of MREF Protocol 58 (Attachment A), the animals were randomly assigned to three weight-homogenized treatment groups of eight animals each and were prepared for treatment prior to study initiation.

2.3 Study Design

The methods detailed in MREF Protocol 58 (Appendix A) for phase one HD dosing only were followed in performing this study. The clipped dorsa of each rabbit was delineated into seven dosing areas of 2.5 cm by 5 cm which were designated as sites A through G as shown below:



The dosing area designated "G" was designated as a TSP-untreated control site. To each of three 2.5 x 5.0 cm dosing areas (B, D, and F) on all 24 animals, a 0.13-mL volume of ICD No. 1536 was applied from a 1 mL syringe (no needle) and spread to a uniform target thickness of 0.1 mm. The control TSP was similarly applied to each of three 2.5 x 5.0 cm dosing areas (A, C, and E) on eight of the 24 animals. The remaining 16 rabbits were not pretreated at sites A, C, and E. Each TSP was allowed to remain undisturbed on the rabbit's back for approximately 60 min prior to HD challenge. Then, 1 µL of HD was applied to each of the TSP-treated test sites and the untreated control sites from a 1 µL gastight syringe equipped with a sharp-tipped needle. Care was exercised to ensure that the TSP layer was not mechanically disturbed in the dosing process. At the protocol-specified decontamination times (i.e., 1, 2, and 4 hr), each of the TSP application sites was decontaminated with a five percent NaOCl solution followed by a distilled water rinse. The TSP-untreated control site "G" was similarly decontaminated immediately prior to the initiation of dermal lesion area evaluation (approximately 20 to 24 hr after HD application).

Twenty-four hours following HD-exposure, lesion lengths and widths were estimated from all HD dose sites, and absolute lesion areas were calculated. Absolute lesion area data from all TSP treated sites were expressed as LARs, i.e., ratios of the lesion area from HD-exposed test sites to the TSP-unprotected, HD-exposed, 24 hr-decontaminated control lesion site on each rabbit (i.e. site "G"). Thus, 24 LARs were estimated for ICD No. 1536, eight for the control TSP, and 16 for no-TSP sites at each exposure period. The control TSP LARs were statistically compared for compatibility with historical control data previously obtained under similar test conditions. The LARs for ICD No. 1536 pretreatment versus those for control TSP treatment were then statistically compared using an unpaired t test with the overall alpha level set at 0.05 in order to contrast the protective efficacy of ICD No. 1536 relative to its previous findings. Finally, the current and historical data for ICD No. 1536 were similarly compared to the historical data for the control TSP and no TSP. To increase the statistical power of the comparison, the historical LARs for the control TSP were used for this comparison if the LARs from the current control TSP sample were found to be compatible with those of the historical data set.

3.0 RESULTS

Areas of lesions resulting from application of 1 µL of HD for individual animals are shown in Table 1. Univariate statistics for absolute HD lesion areas are shown in Table 2. Statistical comparison of current results for ICD No. 1536 efficacy relative to the historical results, the PEG 540 control and no TSP, and associated univariate statistics derived from HD LARs expressed as a percentage of the control dose site on the same rabbit are shown in Table 3. A summary of the historical LARs for the control TSP is shown in Table 4.

The number of rabbits (N = 163) and the mean LARs representing the PEG 540 quality control data base in Table 4 do not match those shown in Table 3 for PEG 540 (N = 139). The reason for this is that during the conduct of Task 89-03 three sets of eight rabbits were found to be outside critical limits and has to be replaced by additional sets (total = 24 rabbits) for a total of 139 rabbits in Table 3. However, all rabbits, even those in sets which exceeded the upper or lower control limits, were retained in the PEG 540 quality control data base (Table 4). One reason for this was that otherwise the critical limits would become closer to the mean as TSP screening proceeded, thereby moving the range for accepting a set of eight animals for a study. Table 4 indicates that LARs for the control TSP fell within the upper and lower critical limits as required for conducting a valid study.

Since the current data from the TSP control sites were found to be statistically compatible with the historical data, statistical comparisons could be made between the historical lesion area data for the control TSP and that of both sets of results for ICD No. 1536. As shown in Table 3, the current assessment of ICD No. 1536, (Lot No. 5256) was completely consistent with the historical assessment. Both sets of data indicated ICD No. 1536 to be statistically superior to both PEG 540 and no TSP pretreatment at the overall 5 percent significance level.

TABLE 1. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RESULTING FROM THREE EXPOSURE PERIODS: INDIVIDUAL RABBIT DATA

Site	G	A		C		E		D		D		F	
TSP/Time	none/24 hr	PEG 540/1 hr		PEG 540/2 hr		PEG 540/4 hr		ICD 1536/1 hr		ICD 1536/2 hr		ICD 1536/4 hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
39390	194	43	22.3	71	36.8	94	48.6	12	6.1	27	14.2	47	24.3
39341	639	198	31.0	286	44.7	286	44.7	306	47.9	377	59.0	403	63.0
39413	302	82	27.1	92	30.5	130	43.0	7	2.3	19	6.3	27	9.1
39202	454	49	10.9	104	22.8	110	24.2	7	1.6	9	2.1	24	5.2
42712	198	25	12.7	55	27.8	71	36.1	5	2.4	13	6.3	27	13.9
39190**													
39414	194	79	40.5	95	49.0	118	60.7	9	4.9	19	9.7	28	14.6
39515	251	85	33.8	130	51.6	179	71.3	9	3.8	63	25.0	75	30.0
TSP/Time	none/24 hr	none /1 hr		none /2 hr		none /4 hr		ICD 1536/1 hr		ICD 1536/2 hr		ICD 1536/4 hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
39221	353	99	28.0	247	70.0	271	76.7	13	3.6	16	4.4	42	12.0
39.84**													
39436	492	188	38.3	283	57.4	318	64.6	49	10.0	85	17.2	85	17.2
39354	224	118	52.6	106	47.4	138	61.8	42	18.9	60	27.0	78	34.7
42711	388	160	41.3	251	64.8	280	72.3	25	6.5	27	7.1	63	16.2
39533	302	174	57.6	247	82.0	194	64.3	27	9.1	69	22.9	165	54.7
42714	254	130	50.9	209	82.1	201	79.0	16	6.2	69	27.2	63	24.7
39224	154	52	33.7	110	71.4	126	81.6	19	12.2	57	36.7	66	42.9
39233	295	184	62.4	253	85.9	255	86.7	55	18.7	104	35.2	78	26.4
39201	295	126	42.7	212	72.0	236	80.0	28	9.6	112	38.1	82	27.7
39350	247	106	42.9	160	64.8	194	78.4	38	15.6	50	20.3	132	53.3
42719	507	264	52.0	253	49.8	377	74.3	99	19.5	85	16.7	170	33.4
39360	577	220	38.1	382	66.1	424	73.5	42	7.3	92	15.9	427	74.0
39239	240	106	44.1	151	62.7	226	94.1	0	0.0	24	9.8	63	26.1
39267	104	85	81.8	86	83.3	123	118.2	47	45.5	20	18.9	71	68.2
39512	259	141	54.5	174	67.0	198	76.4	27	10.6	38	14.5	47	18.2

*Relative area, to G site.
**Died before lesion area was read.

TABLE 2. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS

		Time After Dosing to Decontamination		
TSP		1 hr	2 hr	4 hr
Historical Run	N	24	24	24
ICD No. 1536	MEAN	57.5	79.6	141.3
Lot No. 5256	SD	98.7	111.5	182.4
None	N	31	31	31
	MEAN	131.5	188.6	226.9
	SD	62.9	70.5	78.3
Current Run	N	22	22	22
ICD No. 1536	MEAN	40.1	65.2	102.9
Lot No. 5256	SD	63.5	76.5	108.6
PEG 540	N	139	139	139
	MEAN	165.2	211.6	267.9
	SD	89.4	97.6	124.7

TABLE 3. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

TSP		Time After Dosing to Decontamination 1 hr	2 hr	4 hr	Score**
Historical Run	N	24	24	24	24
ICD No. 1536	MEAN	11.03 A	16.91 A	28.44 A	0.188 A
Lot No. 5256	SD	12.91	18.47	28.84	0.188
Current Run	N	22	22	22	22
ICD No. 1536	MEAN	11.92 A	19.75 A	31.35 A	0.210 A
Lot No. 5256	SD	12.61	13.64	19.83	0.132
PEG 540	N	139	139	139	139
	MEAN	38.04 B	49.37 B	62.47 B	0.500 B
	SD	14.91	15.79	20.91	0.155
None	N	31	31	31	31
	MEAN	46.12 B	67.30 C	78.19 C	0.639 C
	SD	24.89	38.22	25.39	0.282

*Groups having statistically indistinguishable means are indicated with the same letter at each exposure period and Score.
**Mean of 1-, 2-, and 4-hr relative areas, expressed as a fraction.

TABLE 4. HISTORICAL PEG 540 CONTROL VERSUS CURRENT MEAN LESION AREA RATIOS (PERCENT RELATIVE TO NO-TSP CONTROL SITE)

	Time from Exposure to Decontamination					
	1 hr		2 hr		4 hr	
	Historical	Current	Historical	Current	Historical	Current
N	163	7	163	7	163	7
UCL	50.1		64.6		82.3	
Mean	37.2	25.4	48.8	37.6	62.2	46.9
LCL	24.4		33.1		42.1	

UCL = Upper critical limit, mean + 3 standard deviations
LCL = Lower critical limit, mean - 3 standard deviations

4.0 CONCLUSIONS

Current testing of ICD No. 1536 (Lot No. 5256) in the MREF Task 89-03 Phase 1 screen demonstrated complete consistency with the historical assessment in its protective efficacy against HD challenge. ICD No. 1536 (Lot No. 5256) demonstrated significant ($P < 0.05$) protective efficacy against an HD challenge relative to that of the control TSP (PEG 540) and absolute efficacy relative to no TSP.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle Laboratory Notebook No. MREF - 220. Pre-study animal quarantine and observation records are on file at the MREF. All original data, as well as the original copy of this report will be maintained at the MREF until forwarded to the USAMRICD at the conclusion of the contract.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest academic degree of the principal contributors in this study are presented in the following list.

Name	Title	Degree
Garrett S. Dill	MREF Manager	D.V.M.
David W. Hobson	Study Director	Ph.D.
Thomas H. Snider	Biostatistician	B.S.
Peter L. Jepsen	Study Veterinarian	D.V.M.

APPENDIX I

The Efficacy of Lots 5256, 11JAN91BH, and 03JAN91AH of the Topical Skin Protectant, ICD No. 1536, Against a Sulfur Mustard Challenge

SPECIAL REPORT

Contract DAMD17-89-C-9050

on

THE EFFICACY OF LOTS 5256, 11JAN91BH, AND 03JAN91AH OF THE TOPICAL SKIN PROTECTANT ICD NO. 1536 AGAINST A SULFUR MUSTARD CHALLENGE

to

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

February, 1991

Dr. Garrett S. Dill
Dr. David W. Hobson
Mr. Thomas H. Snider

Battelle Columbus Operations
505 King Avenue
Columbus, Ohio 43201-2693

SPECIAL REPORT

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THE EFFICACY OF LOTS 5256, 11JAN91BH, AND 03JAN91AH
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2/25/91
Garrett S. Dill, D.V.M. Date
Principal Investigator and
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2/25/91
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Biostatistician

EXECUTIVE SUMMARY

This report describes the conduct of and provides the results from, a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, "Test Up to Ten Candidate Topical Protectants", which was designed to evaluate and compare the impact of treatment with three specific production lots of a candidate topical skin protectant, Multi-Shield® (ICD No. 1536, manufacturer's Lot Nos. 5256, 11JAN91BH, and 03JAN91AH), to protect against the percutaneous toxicity of sulfur mustard (2,2'- dichlorodiethyl sulfide; HD) using a rabbit model. Lot number 5256 of the candidate skin protectant was previously tested at the MREF, and the results were reported in a letter report to U.S. Army Medical Research and Development Command (USAMRDC) dated 17 August 1990. The purpose of the present study was to assess the efficacy of ICD No. 1536, Lot Nos. 5256, 11JAN91BH, and 03JAN91AH, relative to a control topical skin protectant (TSP) and establish the absolute efficacy of each relative to no TSP. The nominal application thickness of the TSP was 0.1 mm (using an application rate = 0.01 mL/cm^2), and HD applications were fixed at 1.0 μL per dose application site. HD toxicity was assessed following exposures for three different durations (1, 2, and 4 hr) prior to decontamination of each application site. In order to demonstrate and contrast the efficacy of ICD Lot Nos. 5256, 11JAN91BH, and 03JAN91AH relative to a control TSP, dermal lesion areas from sites treated with each lot of the test TSP were calculated and were then statistically compared with pooled historical lesion area data from sites similarly treated with the control (which was a mixture of polyethylene glycols having an average molecular weight of 540 daltons; PEG 540). Results from ICD Nos. 5256 and 11JAN91BH were also compared with data from previous studies conducted using each TSP and under similar test conditions.

Based on statistical comparisons between current and historical results of dermal lesion area ratios obtained for ICD No. 1536, Lot Nos. 5256 and 11JAN91BH, the current data for Lot No. 5256 was statistically equivalent ($P > 0.05$) to historical data, but the current data for Lot No. 11JAN91BH indicated substantially increased efficacy ($P < 0.05$) relative to its performance in the previous study.

Based on statistical comparisons between current results of dermal lesion area ratios obtained for ICD No. 1536, Lot Nos. 5256, 11JAN91BH, and 03JAN91AH, there was no significant difference ($P > 0.05$, adjusted for multiple comparisons) in the protective efficacy noted at any of the HD exposure periods between ICD Lot Nos. 03JAN91AH and 5256, which were statistically better ($P < 0.05$, adjusted for multiple comparisons) than ICD Lot No. 11JAN91BH at the 1- and 2-hr exposure periods. All of the ICD No. 1536 lots were statistically equivalent at the 4-hr exposure period and were statistically better than PEG 540 at all exposure periods.

SPECIAL REPORT

THE EFFICACY OF LOTS 5256, 11JAN91BH, AND 03JAN91AH OF THE TOPICAL SKIN PROTECTANT ICD NO. 1536, AGAINST A SULFUR MUSTARD CHALLENGE

1.0 INTRODUCTION

This report presents the results from a special study conducted under Medical Research and Evaluation Facility (MREF) Task 89-03, entitled "Test Up to Ten Candidate Topical Protectants". In this study, the efficacy of pretreating rabbit skin with specific manufactured lots (Lot Nos. 5256, 11JAN91BH, and 03JAN91AH) of Multi-Shield® (ICD No. 1536), a new topical skin protectant (TSP), was evaluated against percutaneously administered HD. The objectives of this study were to determine whether current results from a topically applied, 0.1 mm-thick, layer of specific lots of ICD Nos. 1536 were consistent with their results from a previous test, and whether there were significant differences detectable between each of the previously tested lots and the results obtained with an additional lot, 03JAN91AH, being evaluated for the first time. The evaluation was conducted using a rabbit model. Efficacy against HD exposure was determined from statistical tests based on the estimation of lesion area ratios (LARs) for each test site. LARs were calculated from the ratio of the HD-induced lesion area from each test site relative to that of a non-pretreated, 24 hr-decontaminated control site on each rabbit. The study was performed in accordance with the phase one provisions for HD testing under MREF Protocol 58.

2.0 METHODS

2.1 Test Materials

HD was obtained from the U.S. Army Medical Research and Development Command (USAMRDC). Chemical purity and appropriate identification were the responsibility of the USAMRDC. The HD used in these studies was identified as being from Lot No. U-6216-CTF-N-1. For quality control purposes, HD lots are periodically assayed for purity and stability at the MREF using an HD standard reference material supplied by the USAMRDC. Based on MREF gas chromatographic

analysis, HD from Lot No. U-6216-CTF-N-1 was found to be approximately 83 percent pure at the time of the study.

The test TSPs, identified as ICD Nos. 1536 (Lot Nos. 5256, 11JAN91BH, and 03JAN91AH), were supplied by the U.S. Army Medical Research Institute for Chemical Defense (USAMRICD). Chemical purity and appropriate identification were the responsibility of the USAMRICD.

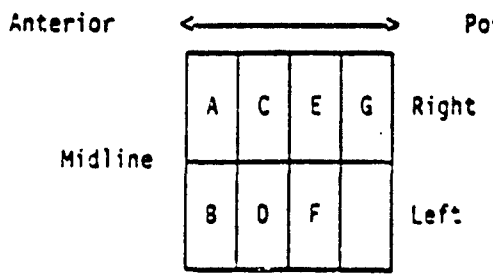
The control TSP was obtained from Union Carbide Corp., and consisted of a proprietary mixture of polyethylene glycol (Carbowax®; Lot No. IS-403051) having an average molecular weight of 540 daltons (PEG 540).

2.2 Animal Model

Forty-eight, specific pathogen free, New Zealand White (albino), male rabbits weighing initially between 2.0 and 4.0 kg in weight were supplied by Hazleton Laboratories. Rabbits were chosen for this study because we have significant prior experience evaluating the percutaneous effects of HD and the application of candidate TSPs with this species. In accordance with the routine provisions of MREF Protocol 58 (Attachment A), the animals were randomly assigned to two weight-homogenized treatment groups of eight animals each per day of dosing and were prepared for treatment prior to study initiation.

2.3 Study Design

The methods detailed in MREF Protocol 58 (Appendix A) for phase one HD dosing only were followed in performing this study. The clipped dorsa of each rabbit was delineated into seven dosing areas of 2.5 cm by 5 cm which were designated as sites A through G as shown below:



The dosing area designated "G" was designated as a TSP-untreated control site. To each of three 2.5 x 5.0 cm dosing areas (B, D, and F) on 24 animals, a 0.13-mL volume of ICD No. 1536, Lot No. 5256 was applied from a 1 mL syringe (no needle) and spread to a uniform target thickness of 0.1 mm. The control TSP was similarly applied to each of three 2.5 x 5.0 cm dosing areas (A, C, and E) on the same 24 animals. In another set of 24 rabbits, Lot Nos. 11JAN91BH and 03JAN91AH of ICD No. 1536 were similarly spread on contralateral sides of the dorsa. Each TSP was allowed to remain undisturbed on the rabbit's back for approximately 60 min prior to HD challenge. Then, 1 µL of HD was applied to each of the TSP-treated test sites and the untreated control sites from a 1 µL gastight syringe equipped with a sharp-tipped needle. Care was exercised to ensure that the TSP layer was not mechanically disturbed in the dosing process. At the protocol-specified decontamination times (i.e., 1, 2, and 4 hr), each of the TSP application sites was decontaminated with a five percent NaOCl solution followed by a distilled water rinse. The TSP-untreated control site "G" was similarly decontaminated immediately prior to the initiation of dermal lesion area evaluation (approximately 20 to 24 hr after HD application).

Twenty-four hr following HD-exposure, lesion lengths and widths were estimated for all HD dose sites, and absolute lesion areas were calculated. Absolute lesion area data from all TSP treated sites were expressed as LARs, i.e., ratios of the lesion area from HD-exposed test sites to the TSP-unprotected, HD-exposed, 24 hr-decontaminated control lesion site on each rabbit (i.e., site "G"). Thus, 24 LARs were estimated for each lot of ICD No. 1536 and the control TSP at each exposure period. The LARs from Lot Nos. 5256 and 11JAN91BH were statistically compared for compatibility with historical data previously obtained under similar test conditions. The LARs for each lot of ICD No. 1536 pretreatment and those for control TSP pretreatment were then statistically compared using an unpaired t test with the overall alpha level set at 0.05. To increase the statistical power of the comparison, the historical LARs for the control TSP were used for this comparison if the LARs from the current control TSP sample were found to be compatible with those of the historical data set.

3.0 RESULTS

Areas of lesions resulting from the application of 1 µL of HD to the individual animals are shown in Table 1. Univariate statistics for absolute HD lesion areas are shown in Table 2. Univariate statistics derived from HD LARs expressed as a percentage of the control dose site on the same rabbit, along with results of contrasts between the three lots of ICD No. 1536 and PEG 540, are shown in Table 3. A summary of the historical LARs for the control TSP is shown in Table 4.

The number of rabbits (N = 187) and the mean LARs representing the PEG 540 quality control data base in Table 4 do not match those shown in Table 3 for PEG 540 (N = 163). The reason for this is that during the conduct of Task 89-03 three sets of eight rabbits were found to be outside critical limits and had to be replaced by additional sets (total = 24 rabbits) for a total of 139 rabbits in Table 3. However, all rabbits, even those in sets which exceeded the upper or lower control limits, were retained in the PEG 540 quality control data base (Table 4). One reason for this was that otherwise the critical limits would become closer to the mean as TSP screening proceeded, thereby reducing the range for accepting a set of eight animals for a study. Table 4 indicates that LARs for the control TSP fell within the upper and lower critical limits as required for conducting a valid study.

Since the current data from the TSP control sites were found to be statistically compatible with the historical data, statistical comparisons could be made between the historical lesion area data for the control TSP and for all lots of ICD No. 1536. The current assessment of ICD No. 1536, (Lot No. 5256) was completely consistent with the historical assessment. However, the current assessment of Lot No. 11JAN91BH indicated it had significantly more efficacy then in its previous assessment.

Using all historical and current standard TSP data and all current ICD No. 1536 data, the multiple comparisons test indicated statistical equivalence between Lot Nos. 5256 and 03JAN91AH at all exposure periods. These lots were significantly better than Lot No. 11JAN91BH (the lot which had shown a poorer performance in previous tests) at the 1- and 2-hr exposure periods, but the three lots were equivalent at the 4-hr exposure period. All three lots of ICD No. 1536 were better than the standard TSP, PEG 540, at all

TABLE 1. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RESULTING FROM THREE EXPOSURE PERIODS: INDIVIDUAL RABBIT DATA

Site	G	A		C		E		B		D		F	
TSP/Time:	none/24 hr	PEG 540/1 hr		PEG 540/2 hr		PEG 540/4 hr		Lot 5256/1 hr		Lot 5356/2 hr		Lot 5256/4 hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
47185	247	75	30.5	138	55.9	163	66.0	16	6.3	28	11.4	49	20.0
40906	236	130	55.0	165	70.0	174	73.7	19	8.0	16	7.0	38	16.3
44988	396	198	50.0	259	65.5	283	71.4	63	15.9	60	15.3	119	30.2
44081	388	141	36.4	176	45.3	212	54.7	16	4.0	38	9.7	71	18.2
44927	495	176	35.6	224	45.2	224	45.2	47	9.5	275	55.6	415	83.8
44172	452	112	24.8	176	38.9	236	52.1	57	12.5	138	30.6	50	11.1
44156	259	123	47.3	143	55.2	151	58.2	16	6.1	106	40.9	75	29.1
44468	327	104	31.7	141	43.3	176	53.8	44	13.5	12	3.6	49	15.1
44235	418	71	16.9	79	18.8	112	26.9	16	3.8	31	7.5	19	4.5
44230	374	94	25.2	163	43.7	271	72.5	24	6.3	94	25.2	173	46.2
44354	271	113	41.7	88	32.5	176	64.9	7	2.6	28	10.4	25	9.3
44118	308	94	30.6	123	39.8	165	53.6	19	6.1	9	3.1	13	4.1
44083	311	102	32.8	123	39.4	110	35.4	31	10.1	43	13.9	33	10.6
44257	452	154	34.1	177	39.1	212	47.0	75	16.7	12	2.6	38	8.5
44330	311	110	35.4	154	49.5	160	51.5	27	8.8	35	11.4	47	15.2
44380	207	95	45.8	123	59.1	153	73.9	20	9.5	16	7.6	38	18.2
44184	330	75	22.9	92	27.9	110	33.3	16	5.0	25	7.6	25	7.6
44163	462	71	15.3	94	20.4	163	35.4	3	0.7	47	10.2	49	10.7
44291	255	55	21.5	75	29.5	78	30.5	28	11.1	38	14.8	55	21.5
41632	283	85	30.0	121	42.8	174	61.4	3	1.1	24	8.3	57	20.0
41672	507	151	29.7	174	34.2	143	28.2	38	7.4	38	7.4	120	23.7
40927	402	121	30.1	163	40.6	224	55.7	38	9.4	38	9.6	24	5.9
6666	537	194	36.1	264	49.1	352	65.5	19	3.5	220	40.9	233	43.4
44311	424	94	22.2	121	28.5	212	50.0	57	13.3	82	19.3	188	44.4

TABLE 1.
(Continued)

TSP/Time:	none/24 hr	11JAN91BH/1hr		11JAN91BH/2hr		11JAN91BH/4hr		03JAN91AH/1hr		03JAN91AH/2hr		03JAN91AH/4hr	
Animal Number	Area (sq. mm)	Area (sq. mm)	RA* (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)	Area (sq. mm)	RA (%)
44205	534	110	20.6	69	12.9	50	9.4	44	8.2	49	9.3	64	11.9
44176	239	79	32.9	25	10.5	118	49.3	7	3.0	44	18.4	133	55.6
47183	763	220	28.8	141	18.5	242	31.7	94	12.3	188	24.7	220	28.8
44185	478	154	32.2	170	35.5	123	25.7	16	3.3	71	14.8	78	16.3
20000	188	49	26.3	71	37.5	55	29.2	9	5.0	42	22.5	86	45.8
40864	524	209	39.9	132	25.2	104	19.8	22	4.2	63	12.0	123	23.4
44194	570	170	29.8	153	26.9	220	38.6	33	5.8	43	7.6	55	9.6
44332	275	75	27.4	85	30.9	134	48.6	12	4.3	75	27.4	60	22.0
44379	267	57	21.2	20	7.4	165	61.8	24	8.8	7	2.6	9	3.5
44310	632	55	8.7	123	19.4	170	26.8	57	8.9	49	7.8	64	10.1
44553	501	121	24.1	153	30.6	55	11.0	38	7.5	44	8.8	24	4.7
44202	259	63	24.2	69	26.7	57	21.8	28	10.9	57	21.8	50	19.4
44362	275	50	18.3	95	34.6	112	40.9	19	6.9	44	16.0	94	34.3
44183	522	123	23.5	207	39.7	101	19.2	50	9.6	242	46.3	78	14.9
44319	415	38	9.1	47	11.4	43	10.4	28	6.8	47	11.4	49	11.9
44388	247	24	9.5	123	49.5	94	38.1	21	8.6	5	1.9	24	9.5
44333	138	7	5.1	24	17.0	44	31.8	3	2.3	7	5.1	31	22.7
44052	295	19	6.4	27	9.3	49	16.8	7	2.4	35	12.0	49	16.8
44355	424	42	10.0	130	30.6	63	14.8	24	5.6	16	3.7	39	9.3
41236	463	75	16.3	515	111.4	164	35.5	38	8.1	75	16.3	47	10.2
44175	377	82	21.7	57	15.0	177	46.9	69	18.3	31	8.3	47	12.5
44416	389	110	28.3	173	44.4	47	12.1	112	28.9	33	8.5	35	9.1
44505	220	19	8.6	49	22.5	95	43.2	19	8.6	27	12.5	47	21.4
44259	412	102	24.8	78	18.9	207	50.3	24	5.7	33	8.0	60	14.7

*Relative area, to G site.
**Died before lesion area was read.

TABLE 2. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS INDEXED BY HD LESION AREAS (sq. mm) RESULTING FROM THREE EXPOSURE PERIODS

		Time After Dosing to Decontamination		
TSP		1 hr	2 hr	4 hr
03JAN91AH	N	24	24	24
	MEAN	33.3	55.3	65.3
	STD	27.0	53.5	44.1
5256	N	24	24	24
	MEAN	29.1	60.5	83.5
	STD	19.4	66.3	90.8
11JAN91BH	N	24	24	24
	MEAN	85.5	114.0	112.0
	STD	58.0	100.7	61.0
PEG 540	N	163	163	163
	MEAN	157.7	202.2	255.7
	STD	85.7	94.8	121.0

TABLE 3. IN VIVO ASSESSMENT OF TOPICAL SKIN PROTECTANTS (TSP) INDEXED BY HD LESION AREAS RELATIVE TO UNPROTECTED SITE (%) RESULTING FROM THREE EXPOSURE PERIODS

ICD Lot No.		Time After Dosing to Decontamination (hr) 1	Grouping*	2	Grouping	4	Grouping	Score**	Grouping
03JAN91AH	N	24		24		24		24	
	MEAN	8.08	A	13.65	A	18.27	A	0.1333	A
	STD***	5.68		9.79		12.49		0.0609	
5256	N	24		24		24		24	
	MEAN	7.97	A	15.58	A	21.57	A	0.1504	A
	STD	4.39		13.57		18.01		0.1018	
11JAN91DH	N	24		24		24		24	
	MEAN	20.74	B	28.60	B	30.57	A	0.2664	B
	STD	9.60		21.05		14.89		0.0919	
PEG 540	N	163		163		163		163	
	MEAN	37.23	C	48.32	C	61.01	B	0.4885	C
	STD	14.41		15.57		20.41		0.1521	

*Means with the same letter are statistically equivalent.
**Mean lesion area ratio across 1-, 2-, and 4-hr exposure periods, expressed as a fraction.
***Standard deviation.

TABLE 4. HISTORICAL PEG 540 CONTROL VERSUS CURRENT MEAN LESION AREA RATIOS (PERCENT RELATIVE TO NO-TSP CONTROL SITE)

	Time from Exposure to Decontamination					
	1 hr		2 hr		4 hr	
	Historical	Current	Historical	Current	Historical	Current
N	187	24	187	24	187	24
UCL	48.8		63.0		80.0	
Mean	36.6	32.6	48.0	42.3	61.0	52.5
LCL	24.5		33.0		42.0	

UCL = Upper critical limit, mean + 3 standard deviations
LCL = Lower critical limit, mean - 3 standard deviations

three exposure periods. The overall index of performance, called Score in Table 3, showed this order of protective efficacy: 03JAN91AH = 5256 > 11JAN91BH > PEG 540.

4.0 CONCLUSIONS

Current testing of ICD No. 1536 (Lot No. 5256) in the MREF Task 89-03 Phase 1 screen demonstrated complete consistency with the historical assessment in its protective efficacy against HD challenge. Lot Nos. 03JAN91AH and 5256 were equivalent in their protective efficacy against HD challenge, which was better than Lot No. 11JAN91BH, despite the significant improvement in the latter relative to previous test results. All three ICD No. 1536 lots were significantly better than PEG 540 against HD challenge.

5.0 RECORD ARCHIVES

Records pertaining to the conduct of this study are contained in Battelle Laboratory Notebook No. MREF - 220. Pre-study animal quarantine and observation records are on file at the MREF. All original data, as well as the original copy of this report will be maintained at the MREF until forwarded to the USAMRICD at the conclusion of the contract.

6.0 ACKNOWLEDGMENTS

The names, role in the study, and highest academic degree of the principal contributors in this study are presented in the following list.

Name	Title	Degree
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